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ATROPINE ABSORPTION AFTER ADMINISTRATION WITH
2-PRALDOXIME CHLORIDE BY AUTOMATIC INJECTOR(U) MADIGAN
ARMY MEDICAL CENTER TACOMA WA K E FRIEDL ET AL DEC 87

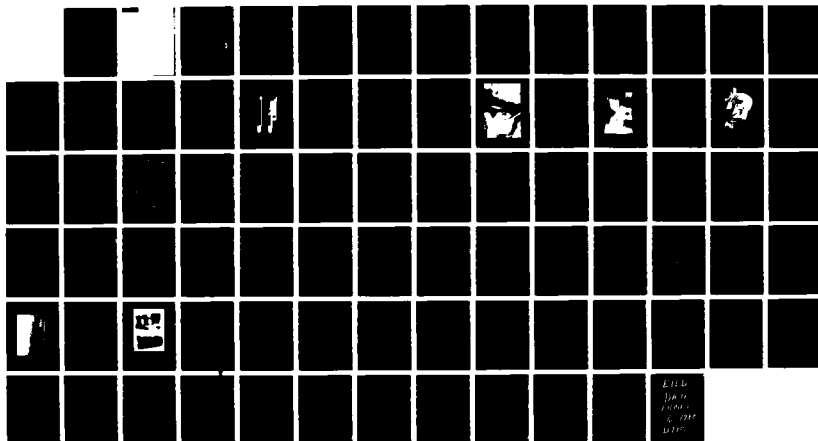
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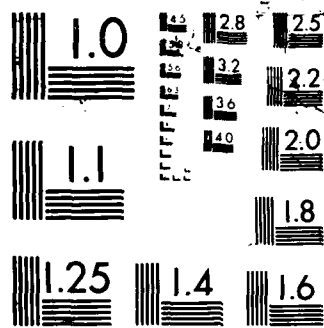
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) We tested the hypothesis that injection of citrated atropine (2 mg/0.7 ml) and pralidoxime chloride (600 mg/2.0 ml) into a single intramuscular site by a multichambered autoinjector (MCP) would deliver atropine as effectively as the currently fielded MARK I device which injects the two drugs into separate intramuscular sites. 20 non-smoking healthy active duty male soldiers (ages 20-30) were injected in a bare upper leg with the MARK I		

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ERRATA/ADDENDA

- p. 9 ADD at the bottom: Atropine content of the injectors was also assayed (n=5) by the same procedures used to measure serum levels in this study. The results (in atropine sulfate equivalents) were by RIA: MARK I, 2.36 ± 0.05 (SEM) mg/dose; MCP, 2.47 ± 0.08 mg/dose, and by RRA: MARK I, 2.27 ± 0.08 mg/dose; MCP: 2.36 ± 0.09 mg/dose).
- p. 33 CHANGE: AUC-90 was not significantly different in the comparison between injectors.
- p. 45 ADD to Figure 14: units on the abscissa are in hours.
- p. 49 ADD to 3rd para: The amount of atropine lost to a chemical suit trousers and BDUs trousers (combined compressed non-pocket thickness: 1.9-2.0 mm) after injection with the atropen (MARK I) was 62 ± 1 ug (RRA, n=3) or < 3.0% of the average amount recovered from injectors not firing through clothing. This indicates that injection through this standard clothing ensemble in the experimental paradigm would not substantially change the outcome.
- p. 52 CHANGE: Thron & Waud (1967) to Thron & Waud (1968).
- p. 53 CHANGE/ADD to 2d para: Endpoints for atropinization are given as "a heart rate of 90-100 heart beats per minutes and a dry mouth and skin" (FM 8-9) and the currently proposed revision of TM 8-285 includes "additional antidote may be given by Combat Lifesaver ...to keep the casualty's heart rate above 90." Seven out of 20 subjects in this study did not reach a peak heart rate of 90 after dosing from one MARK I injector and in the absence of agent. In contrast, a markedly dry mouth was rapidly and consistently observed.

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DEFINITION OF TERMS

- ACCOMMODATION - refers to positive accommodation in this study: the adjustment of the eye for seeing at short distances accomplished by changing the shape of the lens through contraction of the ciliary muscle
- ATROPEN - the smaller of the two component injectors of the MARK I device; delivers approx 2 mg of atropine in 0.7 ml of citrate solution
- ATROPINE - refers to atropine sulfate equivalent units expressed in mass units throughout this report; applied interchangeably to serum atropine levels, to atropine contained in the injector devices (citrated form), and atropine sulfate standard used in both RRA and RIA
- ATROPINE SULFATE - the racemic mixture of d-,l-hyoscyamine which consists of 2 hyoscyamines and 1 sulfate (MW=695); it also usually includes 1 water of hydration (ignored here); for contrast, atropine (free base) MW=290
- ATROPINE (RIA) - serum atropine measured by radioimmunoassay using antibody developed against atropine-BSA immunogen; measures primarily d-,l-hyoscyamine
- ATROPINE (RRA) - serum atropine measured by radioreceptor assay, a bioassay measuring affinity for muscarinic receptor binding; measures primarily l-hyoscyamine and metabolites which compete for muscarinic receptors
- COMBOPEN - the larger of the two component injectors of the MARK I device; delivers approx 600 mg of 2-PAM in 2.0 ml aqueous volume
- MARK I - the currently fielded nerve agent antidote kit composed of a holder/safety cap, atropen, and combopen
- MCP - the "multichambered pen" device; contains 2 plungers inside one chamber separating approx 2 mg of atropine in 0.7 ml volume from approx 600 mg of 2-PAM in 2.0 ml volume; delivers the drugs sequentially through a single needle
- 2-PAM - pralidoxime chloride, an oxime used by the US Army to reactivate acetylcholinesterase after exposure to nerve agents before the agent-acetylcholinesterase bond becomes aged; also referred to as 2-pyridinealldoxime methochloride and 2-pyridinium alldoxime methochloride; distinct from pralidoxime mesylate (methylpyridinium 2-alldoxime methane sulfonate; pralidoxime methylsulfonate)
- SALIVARY SECRETION - refers here to stimulated secretion measured by weight and expressed as percent of the baseline (pre-atropine administration) weight

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COL Paul Knoop (I-Corps Chemical Section), COL Chloupek (I-Corps Surgeon), and LTC Barbeau (9th Division Chemical) were instrumental in generating interest in the project and coordinating our access to dedicated volunteers.

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Finally, no study of this kind is possible without the individual sacrifices made by the study participants, to whom we are indebted.

INTRODUCTION

Chemical warfare, which would include anticholinesterase agents such as soman and VX, is a genuine threat to the U.S. Army (Chemical Warfare Review Commission, 1985). Immediate treatment of soldiers with heroic doses of atropine and an oxime such as pralidoxime chloride (2-PAM) can effectively reverse symptoms and save life (Koelle, 1975). The effectiveness of such an antidote depends on the ease of self-administration by the soldier and the speed of drug absorption. The currently fielded nerve agent antidote kit, the MARK I, is bulky and requires multiple actions to achieve drug infusion at a time when soldier performance is expected to be rapidly deteriorating. It would be convenient to be able to combine these two components into a single injector which would deliver both drugs through a single needle and would further lighten the soldier load. However, problems related to convenience of use are weighed against the second aspect, drug bioavailability. Two previous studies have demonstrated that combining atropine and 2-PAM results in a substantial reduction in the atropine absorption (Sidell, Magness & Bollen, 1970; Sidell, 1974).

Most studies have based their conclusions about atropine action not on measurement of serum atropine levels but on the effect on heart rate. This action is complex and the relation between atropine distribution and heart rate effect is incompletely understood. Intramuscular administration of atropine in a dose of 0.4-0.6 mg may lower heart rate by 4-8 bpm and a larger dose of 2 mg will raise heart rate by 30-40 bpm (Innes & Nickerson, 1975). This biphasic response in heart rate is speculated to result from a shifting balance of opposing effects caused by stimulation of acetylcholine production in the dorsal nucleus, which produces an afferent parasympathetic inhibition of heart rate, and a vagolytic effect at the sinoatrial node (Lowensohn, 1986). Both effects are frequently seen following an intramuscular injection of 2 mg of atropine, with an initial brief reduction in heart rate followed by a prominent and sustained tachycardia.

Sidell (1974) elaborated on the problem of atropine absorption by showing a relationship between delayed heart rate responses and increased osmolarity of the solutions with which atropine was combined. The high osmolarity is a characteristic of the 2-PAM solutions used with the MARK I. A delay in atropine absorption was suggested by a longer bradycardic phase, a delay in the rise to maximal heart rate, and the achievement of lower maximal heart rates following administration of atropine sulfate mixed with 2-PAM (300 mg/ml) compared to atropine sulfate alone (Table 1). The role of osmotic concentration was demonstrated by the time course to a peak heart rate which was slowed for a solution of atropine mixed with 8.5% saline but not for atropine mixed with a more dilute 2-PAM (180 mg/ml).

Table 1. Heart rate data (adapted from Sidell, 1974) showing means (\pm SEM) for baseline heart rate, times to lowest (T MIN) and highest (T MAX) heart rate and amplitude of highest heart rate (HR MAX). Effects of various atropine mixtures are compared to atropine alone.

	Atropine	Atropine & 2-PAM	Atropine & 8.5% saline	Atropine & dilute 2-PAM
Baseline (bpm)	58.0 \pm 0.4	57.6 \pm 0.6	59.5 \pm 0.9	58.9 \pm 1.0
T MIN (mins)	6.4 \pm 0.3	10.8 \pm 0.5 **	10.3 \pm 0.9	7.4 \pm 0.4
T MAX (mins)	43.8 \pm 1.3	60.3 \pm 3.1 **	64.8 \pm 2.5 **	43.6 \pm 2.4
HR MAX (bpm)	35.7 \pm 1.1	26.5 \pm 0.4 **	31.4 \pm 1.3	27.3 \pm 1.4 **

** difference vs atropine alone, paired t test, $p < 0.05$

Another study compared atropine sulfate to atropine sulfate mixed with pralidoxime mesylate (P2S)(Holland, Parkes & White, 1975). No significant differences were established for heart rate response between the two treatments; however, on the basis of a shorter bradycardic phase, the authors concluded that there was a trend to improved absorption when atropine was mixed with the oxime. The data at least equally suggests a trend to impeded absorption very similar to the results of Sidell. This is supported by an apparently lower peak heart rate and a longer time to achieve a peak heart rate following administration of the mixture of atropine and P2S.

It has been suggested that the atropine absorption problem may be caused by sympathomimetic effects (including increased peripheral vascular resistance) which have been documented for 2-PAM (Holland, Parkes & White, 1975). However, these have only been demonstrated to be centrally mediated effects (local effects have not been specifically examined) and any form of combined atropine and 2-PAM treatment would be equally affected (O'Leary et.al. 1962; Zarro & DiPalma, 1965). In a recent study where atropine administered by atropen was compared to the MARK I, the heart rate responses and the increases in serum atropine were identical (Riley & Perkal, 1985); therefore, any systemic effects produced by 2-PAM are inconsequential to atropine absorption.

Although the problem of mixing has not yet been overcome, the MARK I already represents several improvements in the intramuscular administration of atropine. Atropine in citrate buffer acts more rapidly than atropine sulfate and this is thought to be related to a more stable citrate

complex being able to better penetrate lipid membranes (Martin et.al. 1980). By manual injection, atropine in citrate buffer produced peak heart rates at 40 ± 15 (SD) mins compared to 56 ± 20 mins for atropine sulfate. This speed of action is further improved when the citrated form is given by the atropen autoinjector (26 ± 13 mins), confirming earlier findings with automatic injector delivery of atropine sulfate (Martin et.al. 1980; Sidell et.al. 1974). In both of these studies it was shown that the injector contents were more widely dispersed or "sprayed" through muscle tissue compared to the discrete localized deposits which result from administration by manual syringe. This wider dispersion has been attributed to the higher force of delivery into the muscle but part of the enhancement may be due the action of the atropen, which begins drug delivery at the moment the needle emerges from its cartridge. The combopen, which does not deliver drug until the needle is fully extended, has also been shown to improve absorption of drug (2-PAM) (Sidell et.al. 1974) and this implies that there is still some advantage to the greater force of delivery itself.

The human studies which have examined the problem of combining atropine and an oxime have all compared the drugs after administration by manual syringe (Sidell et.al, 1970; Holland, Parkes & White, 1975). It can be logically proposed that the absorption problem which arises when atropine and 2-PAM are mixed in relatively small volume will be lessened or abolished if the solution is more widely distributed through the muscle tissue by the greater force of an autoinjector. Furthermore, there is evidence from animal studies, albeit with human doses administered to beagles, that delivery of the two drugs into a single intramuscular site by multichambered injector produces heart rate responses which are equivalent to those produced by injection with separate autoinjectors similar to the MARK I device (Trouiller & Garrigue, 1986).

This study compares the effectiveness of atropine drug delivery between two autoinjector devices, one with multiple chambers delivering atropine and 2-PAM into one intramuscular site, and the other delivering the two drugs into two separate sites (the MARK I device). Comparison of the two devices was based on four separate physiological endpoints of atropine action (heart rate, salivary secretion, pupil size, and visual accommodation) and also by serum levels achieved for atropine, as measured by radioreceptor assay and by radioimmunoassay. In this study, serum levels and physiological effects achieved by 10 minutes were endpoints considered most critical to the resuscitation of a nerve agent casualty. This study was also designed to include experimental subjects most closely resembling the ultimate end user of the product: physically fit, young soldiers from an infantry division.

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METHODS

1. Medical and legal protection of study volunteers.

a. Study protocol review, approvals & authorization

This study was conducted in accordance with the Nuremberg Code of Ethics in Medical Research, the Declaration of Helsinki, and all pertinent Army Regulations including AR 40-38 (Clinical Investigation Program) and AR 70-25 (Use of Volunteers as Subjects of Research). The plan for this study was reviewed and approved by the Madigan Institutional Review Board (19 September 1986), Clinical Investigation Program Division, Health Services Command (23 January 1987), Human Use Review Office (6 February 1987), and the Human Subjects Research Review Board, OTSG (7 April 1987). The project was conducted with the approval of the Fort Lewis Installation Commander (28 July 1987) and the study did not begin until at least 30 days after submission of an amendment to IND 28301 to the Food & Drug Administration (submitted 1 June 1987).

b. Medical screening and safeguards

In accordance with the research protocol and with a specific installation command directive, a qualified physician was present during all drug administrations and all necessary medical emergency equipment was available in the room. The physician served as the medical monitor with the option to terminate any experiment where the safety of a volunteer was potentially compromised. Before entry to the study each subject submitted to a physical examination and a review of his medical records by the medical monitor. A resting ECG was obtained and reviewed by a cardiologist. Specific disqualifying conditions included cardiac abnormalities, glaucoma, prostate disease, asthma, smoking.

c. Method of recruitment

Twenty subjects were entered from a list of volunteers meeting seven requirements: active duty soldiers, male, not over age 30, non-smokers for at least the past year, within current Army weight standards, no known medical problems, and with the agreement of their commander or supervisor for their study participation. Subjects were entered in the order of the first twenty to present themselves for the medical screening and to be available for the experiments. The volunteers were recruited primarily through a briefing to the 9th Infantry Division chemical officers and non-commissioned officers by the principal investigator. In an informal setting with only the principal investigator, each volunteer gave a signed informed consent which emphasized all known risks of the study, real and theoretical (Appendix Table 1). Each volunteer understood that participation was strictly voluntary and that they could withdraw from further

Table 2. Subject characteristics. Variables influenced by selection criteria included age and BMI. Chemical personnel were specific recruitment targets.

NO.	ETHNIC	EYE COLOR	AGE	WEIGHT (kg)	BMI (kg/m ²)	BSA (m ²)	BODY FAT (%)	HR-1	HR-2	EXERCISE HABITS		
										1	2	3
1	caucasian	blue	20	80.9	25.5	1.99	18.8	69	73	3	7	R/RB
2	so pac isl	brown	24	85.5	25.5	2.09	21.2	50	43	5	6	R/BB
3	filipino	brown	30	72.7	24.3	1.86	16.6	57	60	5	7	R/WL/RB
4	caucasian	blue	23	75.0	23.7	1.93	13.3	62	53	5	7.7	R
5	native am	brown	23	77.7	25.4	1.93	18.8	52	52	6	6	R/WL
6	caucasian	blue	23	68.2	22.3	1.83	11.5	62	56	7	6	R
7	caucasian	hazel	23	70.5	23.5	1.84	14.0	57	73	5	7	R/WL
8	caucasian	brown	28	72.7	23.7	1.84	16.6	74	69	3	8	R
9	caucasian	blue	27	88.6	25.9	2.12	14.9	56	60	5	7	R/WL
10	caucasian	brown	29	68.2	24.2	1.77	23.4	55	52	4	8.5	R
11	asian	brown	25	70.5	23.8	1.83	15.7	67	63	3	8	R
12	caucasian	hazel	25	95.5	28.5	2.19	12.9	70	71	6	6.5	R/WL
13	caucasian	hazel	30	85.9	26.5	2.08	22.0	61	62	3	7.5	R
14	caucasian	blue	21	79.1	23.1	2.01	14.1	48	48	4	7	R
15	hispanic	brown	22	69.1	23.9	1.80	19.3	60	62	7		PT
16	caucasian	blue	30	79.5	24.6	1.99	18.0	55	50	3	8	R/BB
17	caucasian	hazel	27	86.4	24.4	2.12	12.6	48	61	3	7	R/WL
18	caucasian	blue	22	65.9	22.8	1.76	13.0	66	65	5	6	R/WL
19	caucasian	blue	25	72.7	24.3	1.85	13.6	78	63	7		B/WL/RB
20	black	brown	23	87.7	27.7	2.08	16.2	59	50	7	6	R/WL/SW
mean												
std dev												
minimum												
maximum												

NOTES: Eye color: brown with green flecks(hazel).

Body surface area(BSA): from $\log(\text{BSA}) = \log(\text{BW}) * 0.425 + \log(\text{HT}) * 0.725 + 1.8564$

Body fat: from height, neck & waist measurements using the Fitzgerald tables (AR 600-9, Feb 87).

Resting heart rate: baseline from start of first experiment(HR-1) and second experiment(HR-2).

Exercise habits: 1. Days/week of 1 hour or more of exercise, 2. Average running time (min/mile),

3. Principal form(s) of exercise: running(R), unit training(PT), weight lifting(WL),

racquetball(RB), basketball(BB), swimming(SW), biking(B).

study at any time with no adverse actions against them. There was no known peer pressure or command pressure for participation by any individual, but commanders or supervisors of participating soldiers had to agree to allow their soldiers to participate during regular duty hours.

d. Benefits to volunteers

Volunteers were compensated \$200.00 (per soldier per two experiments) for their multiple blood samples (IAW DOD Directive 6000.8, "Funding and Administration of Clinical Investigation Programs" and 24 U.S.C. 30). The chemical officers and non-commissioned officers frequently cited the opportunity to gain first hand experience with the MARK I as their major impetus for volunteering. Nine of the twenty subjects were chemical NCOs or chemical officers.

2. Characteristics of the study subjects.

No subjects were rejected on the basis of the medical screening tests. The characteristics of the subjects are summarized in Table 2. The ethnic distribution included 1 Asian (Korean/American), 1 Black, 1 Filipino, 1 Hispanic, 1 Native American, 1 South Pacific Islander (Tahitian), and 14 Caucasians. Eight subjects had blue eyes, 8 had brown eyes and the other four had hazel eyes (brown with green flecks). Ages ranged from 20 to 30 (mean 25.0 ± 3.1 (SD)). Percent body fat averaged 16.3 ± 3.4 (SD). Resting heart rates were derived from the three baseline samples at the beginning of each of the two experiments and there was no difference in mean starting heart rates between the two experiments. Several of the subjects admitted nervousness (e.g. No. 7 & 8) at the beginning of one or both experiments and the effect on starting heart rate was substantiated by a markedly lower rate in the recovery period at the 9 and 12 hour sampling intervals. The study group averaged 5 days per week of physical training which usually consisted of running and the average running rate was 7 minutes/mile. Ethanol use was reported by all but three of the subjects. Five subjects had consumed moderate quantities of alcohol (1-3 beers or wine coolers) within 24 hours of one or both experiments, 4 subjects reported alcohol use within one week, and 7 subjects reported no alcohol consumption for at least one week before either experiment. No subject reported the use of any significant medications and, as active duty soldiers subject to random drug screens, the expected rate of illicit drug use was between zero and one of the twenty subjects in this study (illegal drug use is detected in approximately 3% of active duty soldiers in the current testing program). No significant abnormalities were revealed for any of the standard clinical serum biochemical parameters (Appendix Table 2).

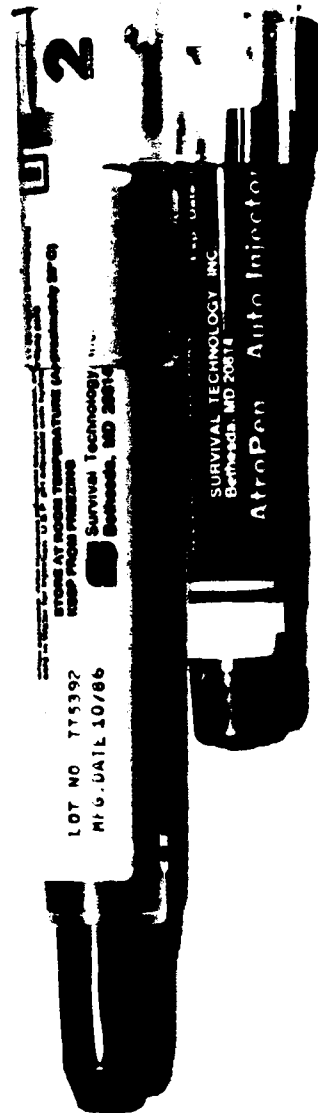
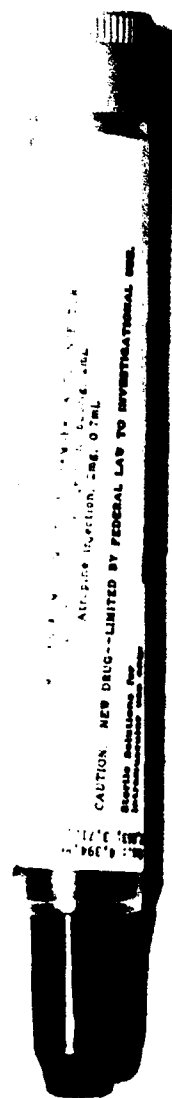


Figure 1. The MARK I (below) and MCP (above) autoinjectors.

3. The automatic injectors & the drug formulations.

The autoinjectors used in this study were both produced by Survival Technology, Inc. (Bethesda, MD). One was the MARK I device which is currently fielded by the Army. This includes two separate nose activated injectors which separate from their safety pins when pulled from a single holder/base. The smaller injector ("atropen") delivers an approximate dose of atropine of 2 mg in a 0.7 ml volume. The larger injector ("combopen") delivers approximately 600 mg of 2-PAM in a 2.0 ml volume. (Lot numbers: atropen, TS4407, exp date 10/91; combopen, TT5392, exp date 10/91).

The multichambered device (referred to in this report as "MCP") was specially produced by Survival Technology, Inc (trade name: "Combopen M.C.") for investigational purposes. The MCP device consists of substantially the same casing as the combopen portion of the MARK I (Figure 1). The same medicaments delivered by the MARK I device are delivered by the MCP device through a single needle. These drugs remain separated until injection by a double plunger system. When activated, the volumes are pushed forward and the atropine is first injected. When the first plunger driving the atropine is all the way forward, the 2-PAM volume is injected past the first plunger and into the needle through three grooved channels in the plastic casing.

The specific composition of each of the injection mixture preparations (used to fill both injection devices) was given by the manufacturer as:

Atropine injection:	2.39 mg/ml	Atropine, USP (base)
	17.81 mg/ml	Glycerine, USP
	4.67 mg/ml	Citric Acid, USP
	4.35 mg/ml	Sodium Citrate, USP
	4.00 mg/ml	Phenol Crystalline, USP
	qs to 1.0 ml	Water for Injection, USP

Pralidoxime chloride		
injection:	330 mg/ml	Pralidoxime Chloride, USP
	20 mg/ml	Benzyl Alcohol, NF
	11.26 mg/ml	Glycine, USP
	qs to 1.0 ml	Water for Injection, USP
	qs to pH 3.0	Hydrochloric Acid, AR

The atropine sulfate equivalent dose delivered by the injectors used in this study were 2.13 mg/dose and 2.32 mg/dose, for the atropen portion of the MARK I and for the MCP, respectively (measured by the manufacturer).

The amount of 2-PAM delivered by each of the two devices was determined by firing either both injectors of the MARK I or the single injector of the MCP through a small hole into a single container. A dilution of the material delivered was then measured in the same assays used for the measurement of

Table 3. Description of drug administrations in terms of experimental injector order, time of injection, leg injected, arm sampled, and weight of injectate.

SUBJ NO.	EXPT NO.	MARK I ORD/HR/LEG/ARM	ATROPEN WEIGHT	COMBOPEN WEIGHT	EXPT NO.	MCP ORD/HR/LEG/ARM	MCP WEIGHT	MARK I TOTAL
1	1	0923 lf lf	0.71	2.10	13	2 0738 rt rt	2.99	2.81
2	9	1 0813 lf lf	0.74	2.17	11	2 0844 rt lf	2.99	2.91
3	11	2 0756 rt rt	0.75	2.19	6	1 0842 lf lf	2.97	2.94
4	10	2 0725 rt lf	0.74	2.15	5	1 0757 lf lf	2.97	2.89
5	6	2 0808 lf rt	0.72	2.15	1	1 1021 rt lf	2.94	2.87
6	2	1 0914 lf lf	0.79	2.19	7	2 0800 rt lf	2.95	2.98
7	6	1 0831 lf rt	0.76	2.20	10	2 0731 rt rt	3.02	2.96
8	11	2 0750 lf rt	0.77	2.17	2	1 0836 rt rt	3.04	2.94
9	4	1 0801 lf lf	0.75	2.19	9	2 0750 rt lf	3.00	2.94
10	9	2 0804 rt lf	0.77	2.20	4	1 0826 lf lf	2.97	2.97
11	11	2 0818 rt lf	0.68	2.03	7	1 0820 lf lf	2.99	2.71
12	5	2 0750 lf lf	0.69	2.14	1	1 1130 rt lf	3.01	2.83
13	7	1 0829 rt lf	0.74	2.19	11	2 0829 lf lf	3.02	2.93
14	1	1 0835 rt rt	0.82	2.06	6	2 0813 lf lf	3.04	2.88
15	7	2 0805 lf rt	0.80	2.19	2	1 0751 rt rt	2.99	2.99
16	8	2 0746 lf lf	0.74	2.15	3	1 0815 rt lf	2.98	2.89
17	9	2 0755 rt lf	0.75	2.17	4	1 0755 lf lf	3.00	2.92
18	2	1 0806 lf lf	0.74	2.18	8	2 0800 rt lf	2.96	2.92
19	8	1 0816 rt rt	0.74	2.20	12	2 0745 lf lf	3.01	2.94
20	3	1 0810 rt rt	0.74	2.21	8	2 0808 lf rt	3.01	2.95
mean			0.75	2.16			2.99	2.91
std dev			0.03	0.05			0.03	0.07
minimum			0.68	2.03			2.94	2.71
maximum			0.82	2.21			3.04	2.99

NOTE: Weights represent the difference of injector weights before and after injection (in grams). 'MARK-I TOTAL' represents the combined weight differences of the atropen and the combopen for comparison to 'MCP WEIGHT' which represents the multichambered injector delivering the two volumes in a single injection. Usually one drop of fluid remained at the tip of each injector. This was usually but not always captured in the postinjection injector weight.

blood levels. This test was repeated for 5 injectors of each type. The 2-PAM dose was 592 ± 2.0 (SEM) mg/dose and 611 ± 7.0 mg/dose, for the MARK I and the MCP, respectively.

The needle length (projecting beyond the injector cartridge) of injectors used in this study was atropen: 2.11 ± 0.01 (SEM) cm, combopen: 2.31 ± 0.02 cm, and MCP: 2.04 ± 0.02 cm. The needle outer diameters were 21 gauge.

4. Data collection procedures.

a. Study design

The study was performed in a double crossover design. Ten of the subjects were injected first with either one of the two autoinjector devices. The subjects were also balanced within any experimental day to study approximately equal numbers of each injector device. Within this experimental blocking, the injector order was randomized to the subjects (schedule in Table 3).

b. Preparation & environment

Volunteers reported to an open bay hospital ward which was reserved for the experiment. They had not eaten or consumed ethanol or caffeine for at least 10 hours prior to the test. They arranged themselves comfortably in a bed which raised the upper body to a 45 degree angle and they remained in this position for the majority of each 12 hour experiment, only leaving their bed to use the restroom after the first few hours of the experiment. Ambient temperature on the ward ranged from 75 to 80 degrees F. Windows were covered to exclude external lighting and internal fluorescent ceiling lights provided 12-16 footcandles of illumination in the vicinity of each subject. Flexible teflon catheters (20 ga, 1.5"; Becton, Dickinson & Co, Rutherford, NJ) were placed in the inner aspect of the arm near the elbow (usually in the antecubital vein) by a skilled technician. Clotting was prevented with periodic infusion of 2-3 mls of heparin flush solution (10 U/ml; LyphoMed; Rosemont, IL).

c. Sampling intervals

Three baseline values were obtained in approximately 10 minute intervals for heart rate, salivary secretion, pupil diameter, and near vision accommodation. A single baseline blood sample was drawn in this period. The individuals were then injected with one the two devices. Sampling was timed from the point of the first injection. In sequence, heart rate, blood sampling, and salivary secretion was collected at 3, 6, 10, 15, 20, 30, 40, 50, 60, 90 minutes and at 2, 2.5, 3, 4, 5, 6, 9 and, 12 hours. Pupil diameters were then measured (except at 15 minutes) and near vision accommodation was measured (except at 3, 6, and 15 minutes).



Figure 2. Method of venous blood collections (see text).



Figure 3. Method of stimulating salivary secretion (see text).

d. Drug administration by automatic injector

The injectors were weighed before and after injection (Table 3). Any injector not falling within one standard deviation of the mean weight of injectors designated for this study was excluded (Appendix Table 3). An area of each volunteer's leg was cleaned with alcohol and the injection(s) was then administered by one of the investigators to the anterio-lateral aspect of the upper leg in the largest available muscle site. The injector(s) was applied perpendicular to the leg and then pushed to activate the needle and initiate the drug delivery. After 10 seconds it was pulled straight out. In the case of the MARK I the second (combopen) injection was made 1-2 inches away from the first injection site in a caudal-rostral plane within 30 seconds.

e. Meals

Meals were served after the 6 hour sample and again before the 12 hour sample. Subjects ate their meals sitting in bed. No stimulants were included. Subjects received 2 cups of milk at each meal and water was available ad libitum. The meals theoretically supplied an average 1380 kcal of useable energy and contained 32-37% fat (Appendix Table 4).

f. Heart rate

Heart rates averaged over a 30 second interval were obtained from three-lead ECGs displayed on a Datascope M/D 3A (Datascope Corp., Paramus, NJ). In some cases a 15 second radial pulse was done instead of relying on the monitor.

g. Blood sampling and handling (Figure 2)

Before blood samples (approx 8 mls) were obtained, a 2.5-3 mls void volume was drawn and discarded. Catheters were then cleared with 2-3 mls of heparin solution (10 U/ml) through a 3-way stopcock arrangement. The blood samples were immediately divided between two 7 ml glass tubes, one containing EDTA, and the other with no preservative. After mixing, the EDTA-treated whole blood was poured directly into polyethylene tubes, tightly capped and immediately frozen in dry ice. After clotting at room temperature for one hour, the second tube (with no preservative) was centrifuged (3000 rpm, 15 minutes) and the serum was removed and aliquoted into polyethylene tubes. The tubes were tightly capped and frozen in dry ice. At the end of the day, all samples were transferred to a freezer and maintained at -70 C until assayed.

h. Salivary secretion (Figure 3)

Salivary secretion was measured after stimulation with a drop of lemon juice. A subject first swallowed all the saliva in his mouth. A drop of pure lemon juice (Minute



Figure 4. Method of pupil measurement (see text).

Maid, Coca-Cola Co, Houston, TX) was placed in the mouth and allowed to remain there for 45 seconds. The contents of the mouth were then collected into a small preweighed plastic cup. This was again weighed and the weight of the saliva was recorded. The mass of saliva was expressed as a percent of the average of three baseline values.

i. Pupil diameters (Figure 4)

Pupil diameters were obtained by matching calibrated semicircles on a rule to each pupil. This was done holding the rule close to the eye without actually touching the eye and taking care not to shade it from ambient light. Diameters were later converted to surface areas (mm²) and normalized to change in surface area from each individual's baseline (average of three pre-injection measurements).

j. Amplitude of accommodation (Figure 5)

The amplitude of accommodation was determined by the proximity method (Stein & Slatt, 1983) using a hand-held slide (R.O. Gulden, Philadelphia, PA). The ruler was held up to each eye (right, then left) at a comfortable arm's length and the slide was gradually brought closer. The point at which the subject reported blurring of small print letters was measured in centimeters and converted to diopters. Each eye was tested individually and the measurements were made with subjects wearing full-distance correction (their usual spectacles).

4. Assays of blood & serum samples and injector contents.

a. Atropine radioreceptor assay (RRA)

Atropine was assayed in serum by a previously described modification (Prete, Hannan & Burkle, 1987) of the radioreceptor method of Metcalfe (1981). Tritiated quinuclidinyl benzylate (specific activity, 30.1 Ci/mmol; New England Nuclear, Boston, MA) was used as the muscarinic agonist. Receptor material was prepared from sheep brain (excluding the cerebellum) by homogenization in 10 volumes of cold 0.25 M sucrose with 10 mM Tris, pH 7.4. This homogenate was processed to yield a suspension of 4.2 mg protein/ml and was stored at -70 C until use. Due to the relatively low muscarinic receptor density of this preparation, the assay was modified from that originally reported for the porcine brain by increasing the volume of receptor in each assay tube to 100 ul. Atropine concentrations were compared to a standard curve constructed using atropine sulfate (Sigma Co, St. Louis, MO) and all results in this report are expressed in mass units (ng/ml) of atropine sulfate. In this experiment the assay had intra- and interassay coefficients of variation of 10% and 11.2%. The limit of detection was 0.3 ng/ml in 100 ul.



Figure 5. Method of measurement of accommodative amplitude (see text).

b. Atropine radioimmunoassay (RIA)

Atropine was assayed in serum at the Walter Reed Army Institute of Research by a previously described and well-validated radioimmunoassay technique (Harrison et.al. 1986). The antibody was developed to measure atropine sulfate and has complete cross-reactivity with atropine and l-hyoscyamine; it does not cross react with tropine or d-,l-tropic acid. Intra- and interassay coefficients of variation are 9.0% and 12.8%, respectively, and sensitivity of the assay is 1 ng/ml of atropine sulfate in a 50 ul sample. This same laboratory performed the serum atropine assay using this technique for an earlier study involving the MARK I (Riley & Perkal, 1985).

c. Pralidoxime chloride (2-PAM)

2-PAM was measured in samples of hemolyzed whole blood by the same method described by Creasey & Green (1959) for measurement of P2S. 2.0 ml samples of whole blood were hemolyzed with 3.8 ml of water and 1 ml of 0.3 M barium hydroxide. 0.33 M zinc sulfate and 0.2 ml 20% NaCl were added and the mixture was centrifuged. The absorption of the alkaline supernatant was then measured at 335 mu (Gilford Response spectrophotometer; Oberlin, Ohio) and compared to a 2-PAM standard curve (2-pyridinealldoxime methochloride; Aldrich Chemical Co, Milwaukee, WI) prepared in deionized water. Baseline samples for each individual were subtracted as background from the other values within experiments. The mean background in the assay was: 1.29 ± 0.40 (SD) ug/ml (n=40). Inter- and intraassay %CV was 3% for 5-30 ug/ml with a sensitivity of 0.5 ug/ml. For purposes of comparison to other studies, some blood concentrations in this report were converted to estimated serum concentrations using the mean hematocrit of subjects in the study ($43.4 \pm 0.6\%$). Since 2-PAM enters erythrocytes (Ellin, Groff & Sidell, 1972), such estimates may be high.

d. Creatine kinase (CPK)

CPK was measured in baseline samples and samples collected 2, 4, and 6 hours after injection using a spectrophotometric method designed for use with an automated clinical analyzer (DACOS, Coulter Electronics, Inc., Hialeah, FL).

5. Data analysis.

Data were analyzed and displayed using a combination of statistics and graphics softwares (BMDP-PC 1987; SPSS-PC; Statgraphics, ver 1.2; Symphony, ver 2.0; GEM Graph). Significance in this study was accepted at the $p < 0.05$ level. For each of the four physiologic endpoints some form of data normalization, comparing the change from individual baselines, was included to minimize the variance produced by individual differences. These variables were analyzed as:

Experimental Measurement	Physiologic Endpoint Analyzed
Heart rate (bpm)	- Heart rate (bpm) - Change in heart rate (bpm)
Salivary mass (g/45 sec)	- Salivary secretion (% baseline)
Pupil diameters (mm) (left & right)	- Pupil diameters (mm) - Change in pupil area (mm ²)
Amplitude of accommodation (cm)	- Accommodation (diopters - D) - Change in accommodation (D)

Each of the basic measurement variables were tested by two way analysis of variance with repeated measures in two factors (injectors and time)(BMDP). In the ANOVAs significant for interactions, specific differences were pursued using paired t-test comparisons of injector means at each time point (SPSS). Means were tabled with standard errors (SEM), as appropriate to small sample t test comparisons. Duncan's multiple range test (BMDP) was used to pinpoint changes over time. Times to maximal change, values at maximal change, and all kinetic parameters were compared by Mann-Whitney test (Statgraphics).

Pharmacokinetic descriptions of the serum atropine and 2-PAM blood levels were attempted using a non-linear curve fitting program designed to describe a two compartment model (BMDP). This method could not satisfactorily resolve the terminal portion of the concentration curves and analyzed only a monoexponential curve, underestimating expected half-times (Fell & Stevens, 1975). Since the purpose of this study was simply to compare the appearance and disappearance of atropine in the serum following injection by the two injector devices, the two kinetic parameters of interest (absorption and elimination half-times) were estimated for each individual graphically (by the method of residuals) (Sidell & Groff, 1971; Trouiller & Garrigue, 1986). Circulating drug concentrations are described in this model of intramuscular injection as:

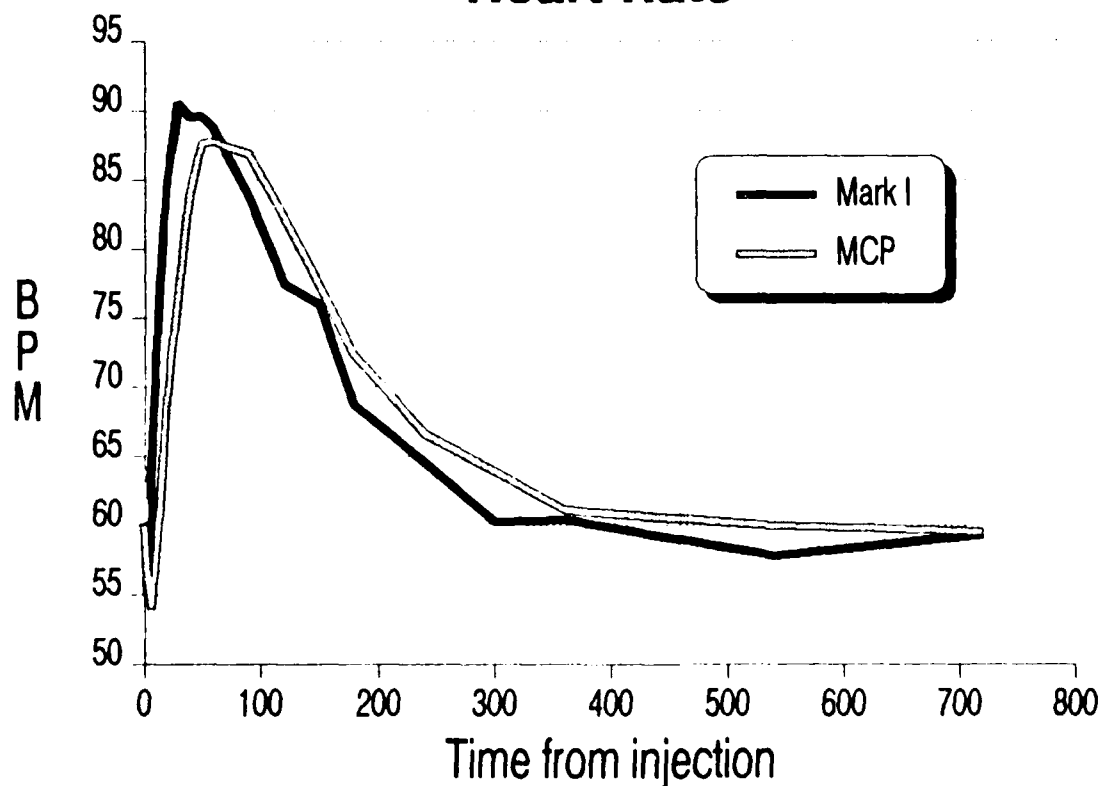
$$C(t) = B e^{-bt} + A e^{-at} - K e^{-kt}$$

B, A, and K are antilog y-intercepts and b, a, and k are slopes derived from three sequential best fit (method of least squares) lines in a plot of ln(concentration) vs time. b is the slope of the line which best fits the terminal points of the concentration curve (usually 5-7 points). k is the slope of the third line which best fits the residuals remaining from the first two linear fits and includes only points up to the maximum (at least three points used). Absorption, distribution, and elimination phase half-times can be estimated as 0.693/k, 0.693/a, and 0.693/b, respectively. AUC was estimated to 90 minutes and to 12 hours by the trapezoidal method (BMDP).

Table 4. Heart Rate. Values are compared between injectors at each time period by paired t test.

Time (mins)	MARK-I		MCP		MEAN DIFF	SEM	t	prob
(bpm)	SEM	(bpm)	SEM					
Baseline	60.2	1.8	60.1	1.9	0.1	1.5	0.06	
3	55.0	2.2	56.4	1.9	-1.4	2.2	-0.65	
6	64.9	2.9	54.0	1.6	10.9	2.5	4.37	0.000
10	72.3	3.5	57.8	2.2	14.5	2.9	4.99	0.000
15	79.3	3.9	63.2	2.7	16.2	2.9	5.60	0.000
20	84.9	3.9	69.2	3.1	15.8	2.9	5.44	0.000
30	90.6	3.1	77.2	4.1	13.4	2.8	4.88	0.000
40	89.6	2.7	83.9	3.4	5.7	1.9	3.04	0.007
50	89.6	2.6	87.7	2.8	2.0	2.0	0.96	
60	88.8	2.5	87.8	2.7	1.1	2.1	0.50	
90	84.4	2.2	86.9	2.6	-2.5	1.5	-1.64	
120	77.5	1.8	82.2	2.5	-4.7	1.9	-2.51	0.022
150	76.2	2.2	77.4	2.4	-1.2	2.0	-0.58	
180	68.7	2.2	72.2	2.1	-3.4	1.9	-1.79	
240	64.6	1.3	66.7	2.0	-2.1	1.7	-1.20	
300	60.3	2.3	63.9	2.0	-3.6	1.8	-2.05	
360	60.5	2.0	61.1	2.1	-0.7	1.8	-0.36	
540	57.9	2.0	60.0	1.6	-2.2	1.6	-1.36	
720	59.1	2.0	59.5	1.8	-0.4	2.0	-0.22	

Heart Rate



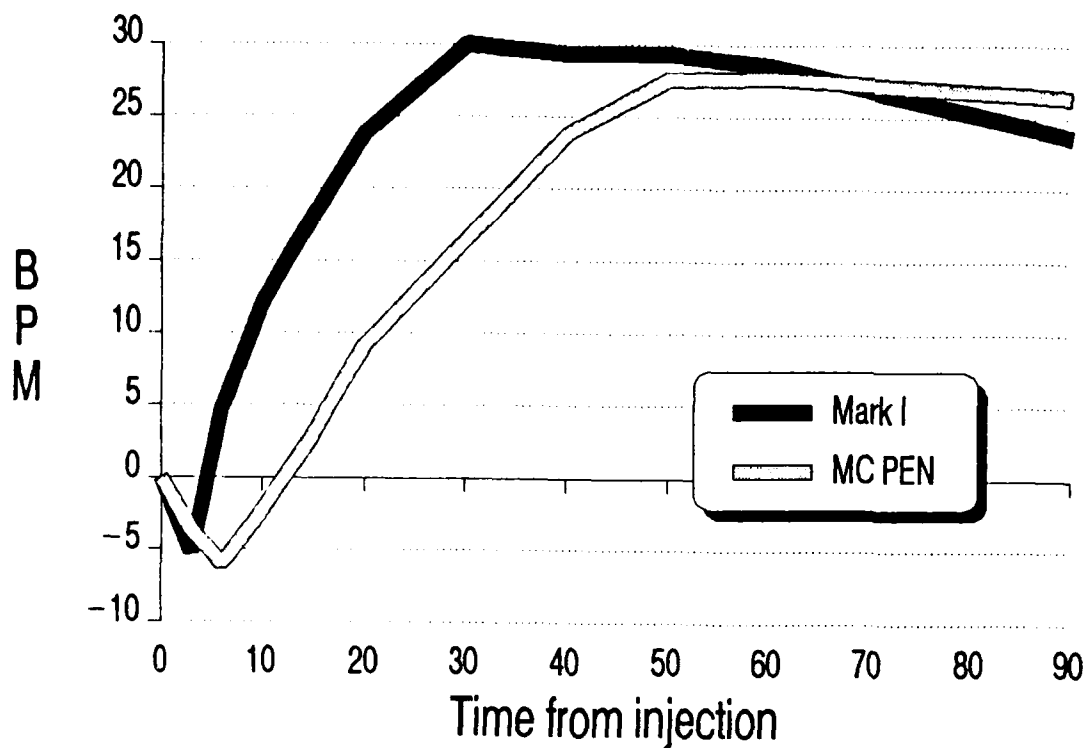


Figure 6. Heart rate expressed as mean change from baseline. Comparison of responses in the first 90 minutes.

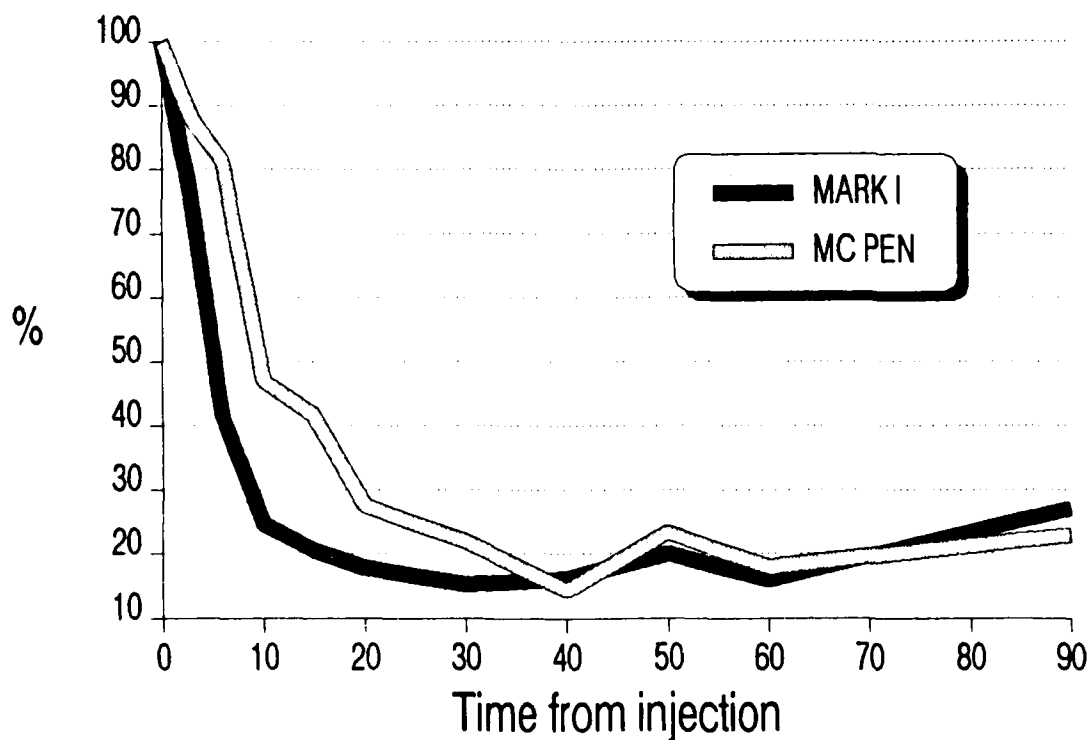


Figure 7. Salivary secretion expressed as mean change from baseline. Comparison of responses in the first 90 minutes.

RESULTS

1. Pharmacodynamics.

a. Heart rate

Heart rate data collected over 12 hours is shown in Table 4. Mean heart rates were significantly elevated from baseline at the 10 minute interval and through the 180 minute interval (MARK I) and from 20 minutes through 180 minutes (MCP) (Duncan's test). The mean peak occurred at 30 minutes (90.6 bpm) for the MARK I compared to 60 minutes (87.8 bpm) for the MCP. These results were no different when the same data was expressed relative to individual baseline heart rates in each experiment (Table 5).

The apparent difference in peaks was not significant and reflected a skewed distribution of individual times to peak. A more appropriate examination of the data, by a distribution free test (Mann-Whitney test) also revealed no significant differences between injectors and gave median times to peak of 40 and 50 minutes for the MARK I and MCP, respectively (Table 13). Individual peak amplitudes were also not significantly different between injectors (MARK I-MCP pairwise: 2.6 ± 1.7 bpm).

The early time course was significantly different between injectors as demonstrated by the difference between mean heart rates at 10 minutes (Table 4). Differences in heart rate response to the two injectors were significant from the 6 minute sampling interval to the 40 minute interval, with a greater response after injection with the MARK I (paired t test; Table 4). From the 6 minute to 30 minute sampling interval the mean difference between injectors for the 20 subjects was greater than 10 bpm. This difference is best illustrated in a view of the first 90 minutes after injection (Figure 6).

b. Salivary secretion

Salivary secretion was significantly reduced from baseline at the 6 minute sampling intervals and did not recover to baseline values until after the 300 min (MARK I) and 360 min (MCP) intervals (Duncan's test). There were significant differences between injectors between the 6 minute to 20 minute sampling intervals, with a much more rapid decline established by 6 minutes following injection with the MARK I (Table 6). The early difference between drug delivery by injector was also reflected in the 10 minute mean difference, with salivary secretion at 24% of baseline following injection with the MARK I compared to 45% of baseline 10 minutes after injection with the MCP (Table 6). The early differences are illustrated in Figure 7. There was no difference in median time to peak change (MARK I: 40 minutes; MCP: 50 minutes) (Table 13) or in minimal levels achieved (Table 14).

Table 5. Change in Heart Rate from baseline. Values are compared between injectors at each time period by paired t test.

Time (mins)	MARK-I		MCP		MEAN			
	(bpm)	SEM	(bpm)	SEM	DIFF	SEM	t	prob
Baseline	0.0	-----	0.0	-----				
3	-5.2	1.1	-3.7	1.6	-1.6	2.1	-0.76	
6	4.7	2.1	-6.1	1.2	10.7	2.5	4.22	0.000
10	12.1	2.7	-2.3	1.7	14.3	2.6	5.52	0.000
15	18.2	2.9	2.6	1.8	15.6	2.5	6.23	0.000
20	23.8	3.1	8.6	2.4	15.3	3.0	5.02	0.000
30	30.0	2.8	16.7	2.9	13.3	2.9	4.52	0.000
40	29.3	2.5	23.8	2.4	5.5	2.1	2.56	0.019
50	29.4	2.6	27.6	1.9	1.8	2.2	0.81	
60	28.6	2.8	27.7	1.9	0.9	2.0	0.43	
90	23.8	2.9	26.4	1.8	-2.6	1.7	-1.52	
120	17.7	2.7	22.9	1.7	-5.2	1.9	-2.73	0.014
150	16.2	2.7	17.5	1.9	-0.7	2.1	-0.31	
180	7.9	2.7	11.5	1.6	-3.6	2.1	-1.69	
240	4.4	2.3	6.6	1.8	-2.2	2.1	-1.07	
300	0.1	2.3	3.8	1.3	-3.8	2.2	-1.74	
360	0.2	2.3	1.0	1.5	-8.3	2.5	-0.33	
540	-2.4	2.0	-0.1	1.8	-2.3	2.2	-1.06	
720	-0.1	2.6	0.2	1.6	-0.4	2.3	-0.17	

Change in Heart Rate from baseline

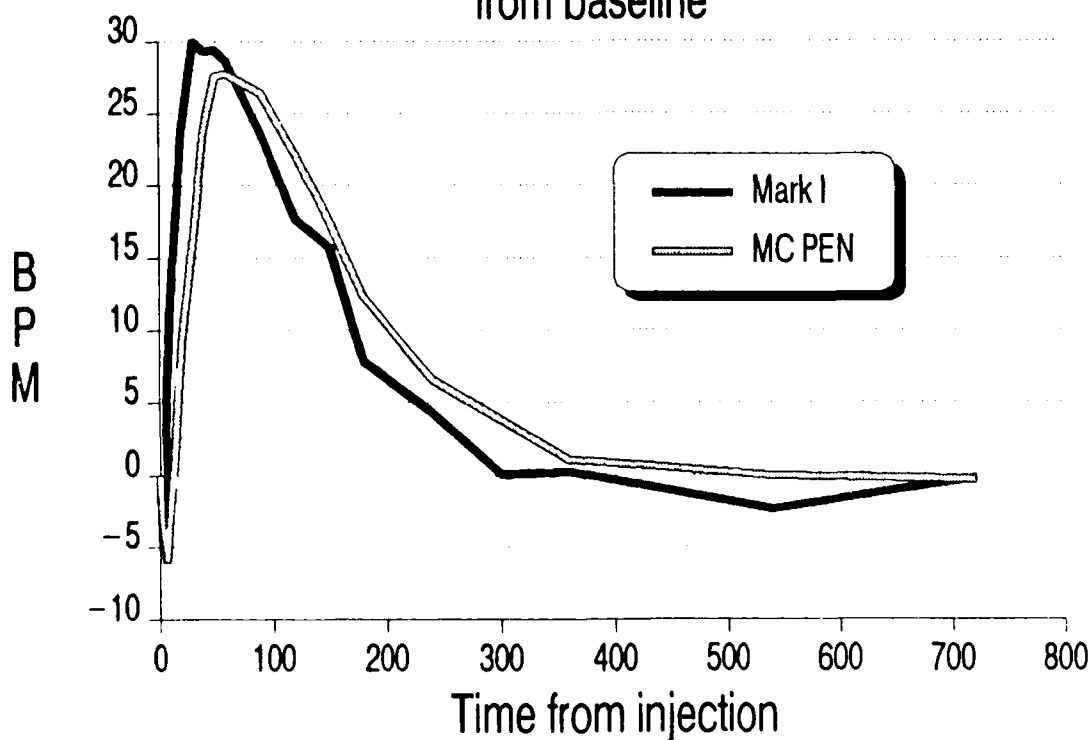


Table 6. Mean salivary secretion at each time period. Values are compared between injectors at each time period by paired t test.

Time (mins)	MARK-I		MCP		MEAN		t	prob
	(%)	SEM	(%)	SEM	DIFF	SEM		
Baseline	100.0		100.0					
3	74.1	9.4	85.2	9.1	-11.1	13.1	-0.85	
6	39.8	5.9	83.0	12.7	-43.3	11.0	-3.95	0.001
10	23.6	5.1	44.7	6.4	-21.1	6.1	-3.47	0.003
15	19.5	3.6	41.8	7.3	-22.3	6.5	-3.44	0.003
20	17.8	3.7	27.7	4.4	-9.8	3.7	-2.66	0.015
30	15.2	2.1	19.5	3.3	-4.2	4.0	-1.05	
40	16.2	3.2	14.4	2.2	1.8	4.3	0.41	
50	18.8	3.3	23.3	3.9	-4.5	5.1	-0.88	
60	15.9	2.3	18.4	3.7	-2.4	4.8	-0.50	
90	26.6	3.9	22.9	3.3	3.7	5.2	0.71	
120	29.8	3.3	31.8	4.7	-2.0	5.4	-0.38	
150	37.7	5.7	34.2	4.1	3.5	6.9	0.51	
180	44.7	5.4	37.0	5.0	7.7	7.1	1.08	
240	57.7	6.1	52.9	8.1	4.7	11.0	0.43	
300	83.2	10.9	66.8	7.9	16.3	11.5	1.42	
360	90.6	10.6	82.3	10.7	8.3	14.9	0.56	
540	92.7	8.2	110.7	14.6	-18.0	17.4	-1.04	
720	123.2	11.5	113.6	11.8	9.6	15.8	0.61	

Salivary Secretion Percent of Baseline

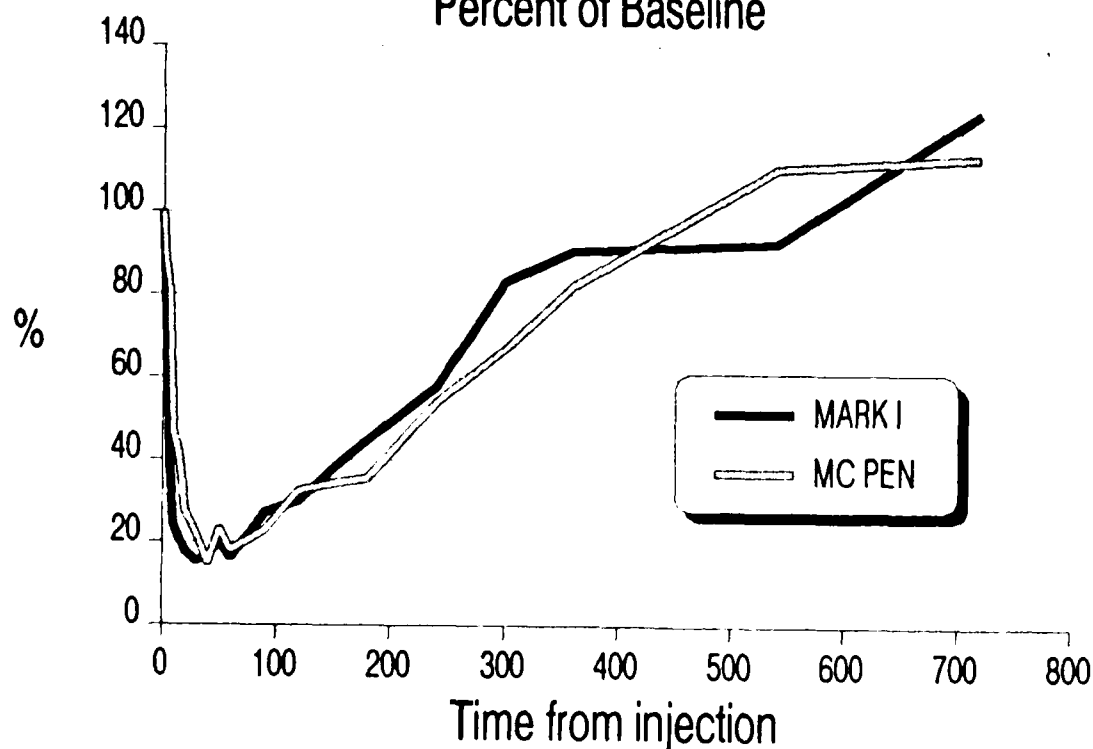


Table 7. Mean pupil diameter (right eye). There were no significant differences between the two injectors (by ANOVA).

Time (mins)	MARK-I (mm) SEM		MCP (mm) SEM		MEAN DIFF SEM		t	prob
Baseline	4.0	0.1	4.2	0.2	-0.3	0.2		
3	4.1	0.2	4.1	0.2	-0.1	0.2		
6	4.1	0.2	4.2	0.2	-0.1	0.2		
10	4.1	0.2	4.2	0.2	-0.1	0.2		
20	4.5	0.2	4.3	0.2	0.2	0.1		
30	4.7	0.2	4.3	0.2	0.5	0.2		
40	5.0	0.2	4.6	0.3	0.4	0.2		
50	5.2	0.2	4.7	0.2	0.5	0.1		
60	5.3	0.2	4.9	0.2	0.4	0.2		
90	5.6	0.2	5.3	0.2	0.3	0.2		
120	5.6	0.2	5.5	0.3	0.1	0.2		
150	5.5	0.2	5.7	0.3	-0.2	0.2		
180	5.6	0.2	5.5	0.2	0.1	0.2		
240	5.8	0.2	5.6	0.2	0.2	0.2		
300	5.5	0.3	5.5	0.2	0.0	0.2		
360	5.2	0.2	5.2	0.3	-0.1	0.3		
540	5.0	0.2	5.4	0.3	-0.4	0.3		

Table 8. Mean pupil diameter (left eye). There were no significant differences between the two injectors (by ANOVA).

Time (mins)	MARK-I (mm) SEM		MCP (mm) SEM		MEAN DIFF SEM		t	prob
Baseline	4.0	0.1	4.3	0.2	-0.3	0.2		
3	4.3	0.2	4.2	0.2	0.1	0.2		
6	4.4	0.2	4.4	0.2	0.1	0.2		
10	4.3	0.2	4.3	0.2	0.1	0.2		
20	4.6	0.2	4.3	0.2	0.2	0.1		
30	4.8	0.2	4.3	0.2	0.5	0.2		
40	5.1	0.2	4.8	0.2	0.3	0.2		
50	5.3	0.2	4.8	0.2	0.5	0.2		
60	5.3	0.2	5.0	0.2	0.3	0.2		
90	5.5	0.2	5.2	0.2	0.3	0.2		
120	5.7	0.3	5.5	0.3	0.2	0.2		
150	5.7	0.3	5.5	0.3	0.2	0.2		
180	5.8	0.2	5.4	0.2	0.4	0.2		
240	5.8	0.2	5.5	0.2	0.3	0.2		
300	5.5	0.3	5.5	0.2	0.0	0.2		
360	5.3	0.2	5.3	0.2	0.1	0.3		
540	5.0	0.2	5.3	0.3	-0.3	0.3		

c. Pupil size

Pupil diameters were significantly larger than baseline at 30 minutes (right and left eyes, MARK I) or by 60 minutes (right and left eyes, MCP) and remained enlarged through the last sampling interval at 12 hours (Duncan's test). There were no statistically significant differences in the behavior of pupil diameters when compared by injector (Appendix Tables 6-4, 6-5; Table 7 & Table 8). No anisocoria (>1 mm difference) was observed in any subject.

Baseline diameters ranged from 3-7 mm between individuals and this wide interindividual variation masked significant differences between injectors. Expressed as change in pupil area, differences between injectors were demonstrated from 30 to 60 minutes (Tables 9 & 10, Figure 8).

d. Amplitude of accommodation

Accommodation was significantly reduced over time after atropine administration (ANOVA, Appendix Tables 6-6, 6-7) but no individual sampling interval could be pinpointed as different from baseline. There were no differences between injectors (Table 11 & Table 12).

Expressed in terms of individual baseline values (change in accommodation), there was little improvement in the variance and this analysis was not pursued.

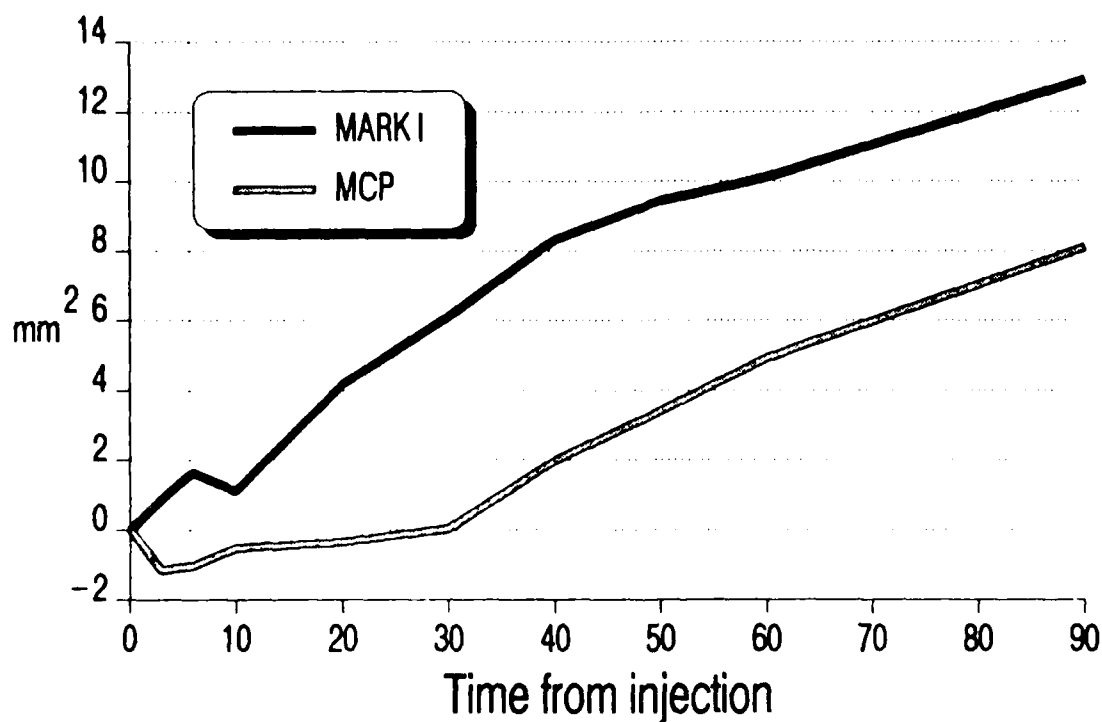


Figure 8. Mean change in pupil area (right eye). Comparison of responses in the first 90 minutes.

Table 9. Mean change in pupil area (right eye). Values are compared between injectors at each time period by paired t test.

Time (mins)	MARK-I (mm ²) SEM		MCP (mm ²) SEM		MEAN DIFF SEM		t	prob
Baseline								
3	0.4	0.4	-1.1	1.0	1.5	1.3	1.19	
6	0.9	0.8	-1.0	1.4	2.0	1.8	1.10	
10	1.2	1.1	-0.7	1.1	1.8	1.6	1.17	
20	3.8	1.2	-0.3	1.4	4.2	2.3	1.82	
30	5.4	1.0	-0.4	0.9	5.8	1.4	4.04	0.001
40	7.1	1.4	2.0	1.8	5.1	2.1	2.41	0.028
50	9.4	1.7	3.3	1.2	6.2	2.1	2.97	0.008
60	10.1	1.7	4.9	1.6	5.2	2.2	2.35	0.03
90	13.0	2.2	8.1	1.8	4.9	3.0	1.66	
120	12.7	1.7	10.6	2.6	2.1	2.8	0.74	
150	12.3	1.9	11.5	2.2	0.8	2.5	0.31	
180	12.9	1.7	9.9	2.1	3.0	2.6	1.16	
240	14.4	1.9	10.5	1.9	3.9	2.6	1.49	
300	11.9	2.0	9.5	1.9	2.4	2.7	0.86	
360	9.3	1.7	7.6	1.7	1.7	2.5	0.68	
540	7.7	1.7	9.2	2.0	-1.5	2.5	-0.59	

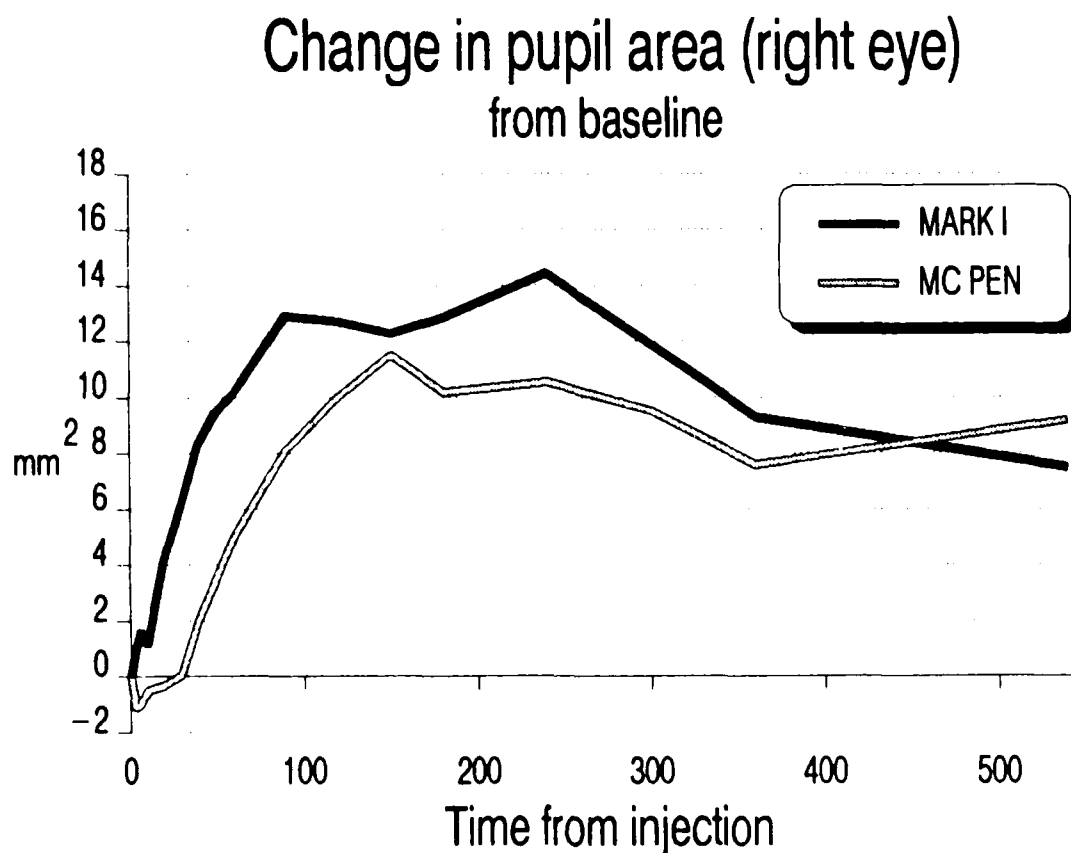


Table 10. Mean change in pupil area (left eye). Values are compared between injectors at each time period by paired t test.

Time (mins)	MARK-I (mm ²) SEM		MCP (mm ²) SEM		MEAN DIFF SEM		t	prob
Baseline								
3	1.5	0.6	-0.9	0.9	2.4	1.1	2.15	0.046
6	2.7	1.3	-0.3	1.1	3.0	1.4	2.11	
10	1.9	1.2	-0.2	1.5	2.1	1.2	1.70	
20	3.8	1.1	-0.2	0.9	3.9	1.5	2.63	
30	5.5	1.0	0.0	1.0	5.5	1.2	4.73	0.000
40	7.7	1.8	3.7	1.8	4.0	1.9	2.08	
50	9.6	1.9	3.3	1.2	6.3	1.9	3.30	0.004
60	9.5	1.8	5.3	1.3	4.2	2.0	2.09	
90	11.5	2.0	6.5	1.5	4.9	2.2	2.24	0.038
120	13.3	1.8	9.9	2.1	3.4	2.1	1.61	
150	13.7	2.1	9.9	2.2	3.8	2.3	1.64	
180	14.5	2.1	9.0	1.7	5.5	2.5	2.22	0.039
240	13.6	1.8	9.3	1.6	4.4	1.8	2.45	0.024
300	11.4	2.0	8.9	1.5	2.6	2.5	1.03	
360	9.7	1.8	7.3	1.6	2.4	2.3	1.04	
540	7.1	1.4	7.9	2.1	-0.8	2.3	-0.36	

Change in pupil area (left eye) from baseline

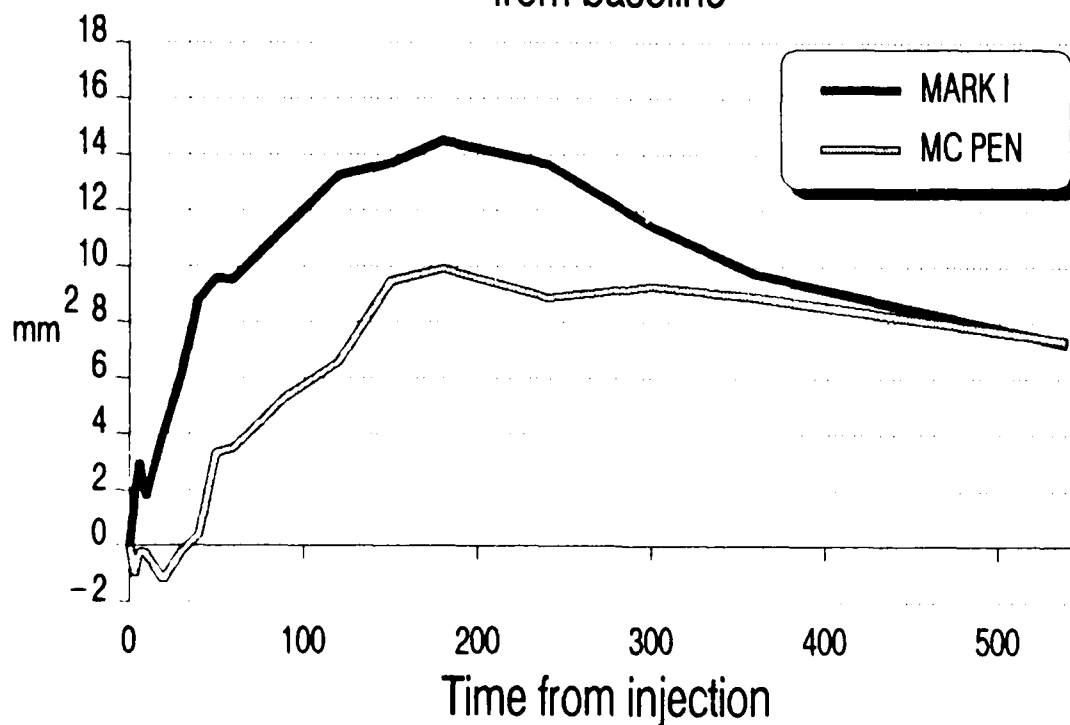


Table 11. Visual accommodation (right eye). There were no significant differences between injectors (ANOVA).

Time (mins)	MARK-I (diop)	SEM	MCP (diop)	SEM	MEAN DIFF	SEM	t	prob
Baseline	8.9	0.4	8.9	0.4	-0.0	-0.20		
10	8.4	0.4	8.1	0.4	0.3	1.42		
20	8.1	0.4	8.3	0.4	-0.2	0.31		
30	8.1	0.6	8.0	0.6	0.1	0.49		
40	7.5	0.5	7.6	0.6	-0.1	0.44		
50	7.2	0.5	7.8	0.6	-0.6	0.38		
60	7.5	0.6	7.6	0.5	-0.0	0.28		
90	7.1	0.5	7.5	0.5	-0.4	0.32		
120	7.2	0.6	7.9	0.5	-0.8	0.25		
150	7.1	0.5	7.4	0.4	-0.3	0.24		
180	6.9	0.5	7.7	0.5	-0.8	0.35		
240	6.6	0.3	7.3	0.4	-0.6	0.30		
300	6.8	0.4	7.4	0.5	-0.6	0.30		
360	6.7	0.3	7.3	0.4	-0.6	0.24		
540	7.4	0.4	7.7	0.5	-0.2	0.26		
720	7.5	0.4	7.5	0.5	-0.0	0.25		

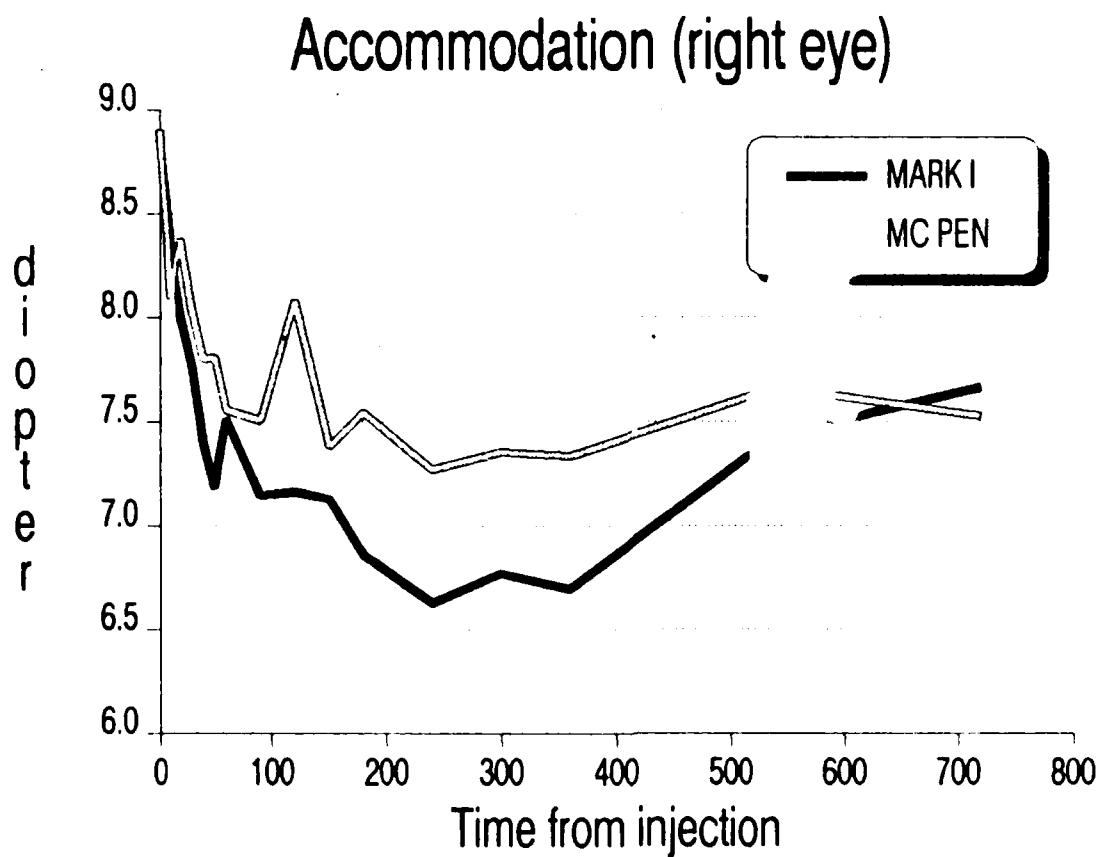


Table 12. Visual accommodation (left eye). There were no significant differences between injectors (ANOVA).

Time (mins)	MARK-I (diop) SEM	MCP (diop) SEM	MEAN DIFF	SEM	t	prob
Baseline	8.9 0.4	8.9 0.5	0.0	0.25		
10	8.3 0.5	7.9 0.6	0.4	0.31		
20	8.2 0.5	7.8 0.5	0.4	0.40		
30	8.0 0.7	7.8 0.7	0.2	0.47		
40	7.2 0.5	7.6 0.7	-0.4	0.43		
50	6.9 0.6	7.6 0.6	-0.7	0.40		
60	7.1 0.6	7.3 0.6	-0.2	0.33		
90	7.2 0.6	7.1 0.6	0.1	0.40		
120	6.9 0.6	7.8 0.5	-0.9	0.34		
150	7.1 0.7	7.2 0.5	-0.1	0.38		
180	6.6 0.5	7.3 0.5	-0.7	0.36		
240	6.6 0.5	7.0 0.5	-0.4	0.34		
300	6.4 0.5	7.3 0.6	-0.9	0.44		
360	6.6 0.4	7.6 0.6	-1.0	0.41		
540	7.5 0.4	7.5 0.5	0.1	0.27		
720	7.4 0.5	7.5 0.5	-0.0	0.28		

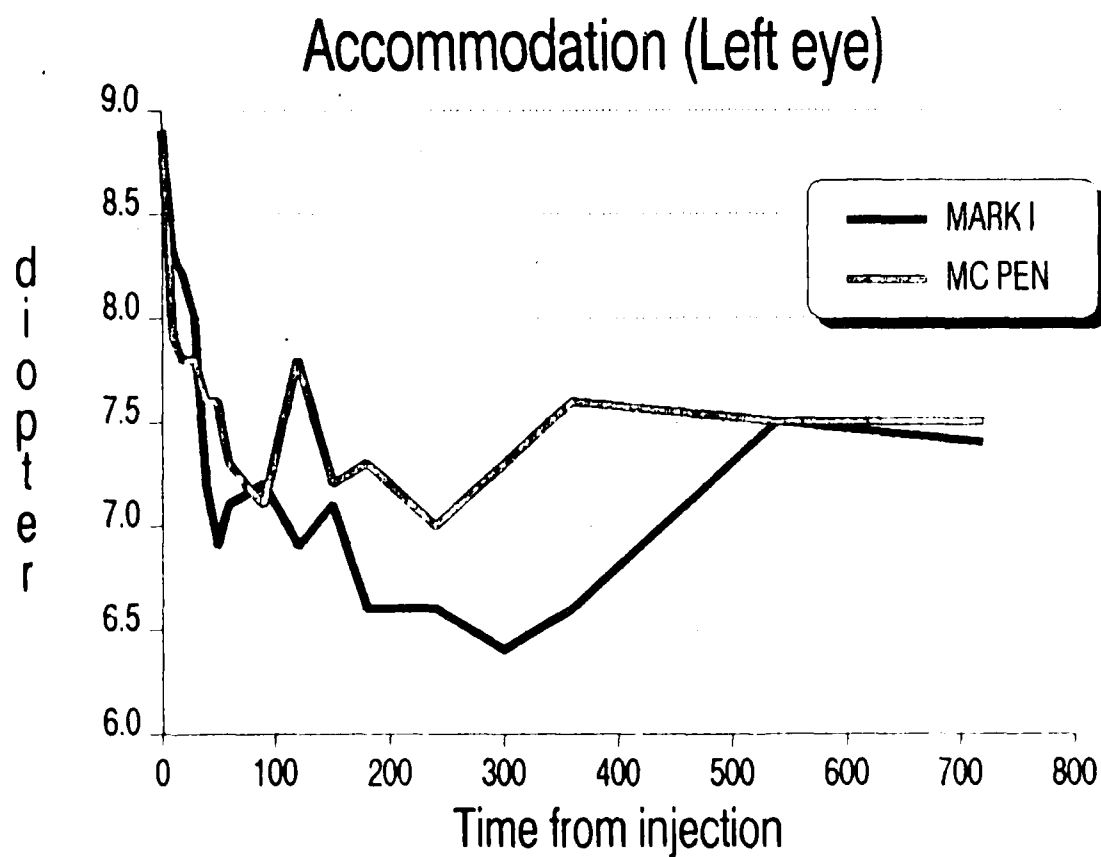


Table 13. Frequency distributions of individual maximal changes (by time interval)
Comparisons are shown between injectors using Mann-Whitney-Wilcoxon test.

TIME INTERVAL	HEART RATE		CHANGE IN SALIVA		R PUPIL DIAM.		R ACCOMM	
	MARK-I	MC PEN	MARK-I	MC PEN	MARK-I	MC PEN	MARK-I	MC PEN
3								
6								
10	*		*		*		*	
15	***							
20	***		***				*	
30	***	**	***	***				*
40	M**	*****	M***	*****			*	*
50	**	M*****		M**	*		*	*
60	***	***	*****	*****	*			
90	**	***	*	**	***		*	****
120	*				***		**	
150					M**		***	
180					**		*	*
240					***		M*	M*
300					**		M**	***
360					*		**	**
540					*		****	*
Median	40	50	40	50	120	120	150	120
z score	-0.053		-0.054		-0.043		0.196	
p value	0.958		0.958		0.967		0.845	

Table 14. Individual greatest changes (minimum or maximum) for physiological endpoints. Median values for injectors are compared by Mann-Whitney-Wilcoxon.

SUBJ	HEART RATE		CHG IN HR		SALIVARY %		PUPIL DIAM		CHG P AREA		ACCOMMODAT	
	MARK	MCP	MARK	MCP	MARK	MCP	MARK	MCP	MARK	MCP	MARK	MCP
1	101	103	32.3	29.7	6.7	8.2	7.0	7.0	27.9	11.8	6.5	7.4
2	100	90	56.7	40.0	16.9	0.0	5.0	4.0	7.1	2.0	7.4	8.3
3	85	85	22.0	27.7	0.8	4.8	5.0	5.0	9.1	6.0	7.1	6.7
4	91	93	38.0	31.3	23.8	0.0	6.0	5.0	18.7	12.6	4.4	5.3
5	80	87	26.7	35.3	32.4	0.0	6.5	8.0	20.6	37.7	6.9	6.3
6	95	90	31.0	34.7	7.0	13.4	8.0	8.0	31.9	11.8	3.6	3.6
7	101	100	42.3	27.3	7.7	5.9	6.5	5.5	20.6	13.2	6.9	6.7
8	100	111	30.0	37.0	8.2	4.6	6.0	6.0	19.5	18.7	7.1	6.7
9	79	75	18.3	14.7	8.2	12.9	5.5	5.5	11.2	12.2	3.3	4.9
10	88	89	35.3	34.3	22.0	10.9	6.0	7.5	16.7	28.3	3.9	2.0
11	98	108	35.7	41.3	6.0	6.3	6.0	6.0	18.7	13.5	5.4	5.3
12	104	96	32.7	23.3	7.0	26.5	6.5	7.0	17.3	24.8	7.7	9.1
13	96	88	35.3	26.3	7.5	9.5	7.5	6.5	34.6	21.6	4.6	4.6
14	83	76	35.0	27.7	2.8	20.5	8.0	8.0	35.5	27.9	7.4	6.9
15	110	104	48.7	43.0	9.3	1.1	6.0	6.0	14.6	12.4	5.7	7.1
16	76	85	25.0	32.0	0.0	4.1	6.0	7.0	18.7	30.6	4.8	6.9
17	71	67	10.0	18.3	1.7	12.7	6.5	7.0	8.0	16.1	5.6	5.6
18	113	104	46.7	38.7	0.0	4.8	6.0	6.5	15.7	20.6	3.6	4.4
19	96	82	20.0	19.0	1.5	6.0	6.0	6.0	19.5	17.7	5.7	5.7
20	104	87	44.7	37.3	4.3	13.4	4.5	4.5	5.3	8.0	8.7	10.0
MED	96.0	89.5	33.9	31.7	7.0	6.2	6.0	6.3	18.7	14.8	5.7	6.5
P	NS		NS		NS		NS		NS		NS	

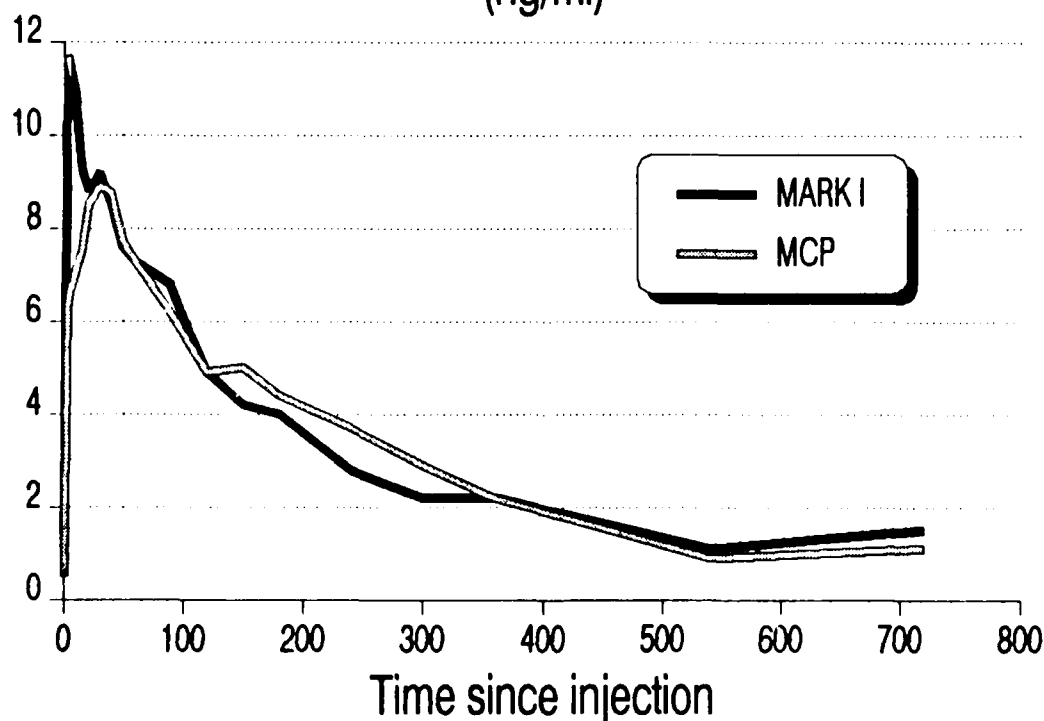
NOTE: heart rate(bpm); heart rate change from baseline (bpm); percent of baseline salivary secretion; right eye pupil diameter(mm); right eye change in pupil area (mm); right eye accommodative amplitude(diopters).

Table 15. Mean serum atropine (RRA) concentrations (a.sulfate equiv) compared between injectors at each time period by paired t test.

Time (mins)	MARK-I		MCP		MEAN		t	prob
	ng/ml	SEM	ng/ml	SEM	DIFF	SEM		
Baseline	0.5	0.2	0.6	0.3	-0.2	0.4	-0.40	
3	11.7	1.9	6.3	1.2	5.4	1.8	3.08	0.006
6	11.4	1.3	6.7	1.0	4.7	1.5	3.25	0.004
10	10.9	1.2	7.1	0.9	3.7	1.4	2.72	0.014
15	9.3	1.1	7.5	1.0	1.8	1.2	1.57	
20	8.8	0.9	8.5	1.0	0.3	1.3	0.21	
30	9.2	1.1	8.9	0.7	0.3	1.3	0.23	
40	8.5	1.0	8.8	1.3	-0.3	1.8	-0.16	
50	7.6	0.9	7.7	0.7	-0.1	1.2	-0.09	
60	7.3	0.7	7.3	1.0	0.0	1.1	-0.03	
90	6.8	0.9	6.2	0.7	0.6	1.2	0.47	
120	4.9	0.7	4.9	0.6	0.0	1.0	0.00	
150	4.2	0.6	5.0	0.6	-0.8	1.1	-0.76	
180	4.0	0.6	4.4	0.7	-0.5	1.0	-0.44	
240	2.8	0.5	3.7	0.6	-1.0	0.9	-1.12	
300	2.2	0.5	2.9	0.6	-0.7	0.9	-0.76	
360	2.2	0.5	2.2	0.4	0.0	0.8	0.00	
540	1.1	0.3	0.9	0.3	0.2	0.4	0.43	
720	1.5	0.3	1.1	0.3	0.4	0.6	0.64	

Serum Atropine Levels (RRA)

(ng/ml)



2. Pharmacokinetics.

a. Serum atropine (by radioreceptor assay)

Serum atropine levels were significantly elevated above baseline from 3 minutes to at least 150 minutes (Duncan's test) and these levels were different between injector from 3 to 10 minutes (Table 15). In the first sampling interval (3 minutes), 7 out of 20 subjects peaked and 15 had peaked by 10 minutes following injection by the MARK I. In contrast, 4 out of 20 subjects peaked at 3 minutes and only 6 had peaked by 10 minutes after injection with the MCP. The median time to peak was 6 minutes and 25 minutes for the MARK I and MCP, respectively. This was a significant difference (Table 16). The median peak level achieved was not significantly different between injectors. AUC-90 (Area under the curve to 90 minutes) was significantly different but AUC-12 hours was not (Table 16). The early differences between injectors are illustrated in Figure 9.

The effect of body weight, lean body mass, fat mass and body surface area was tested in a stepwise multiple regression procedure against serum atropine. Body weight was selected as the most significant covariate but this accounted for less than 3% of the variance. Accordingly, no adjustment for body size or body composition was made to the data in this study.

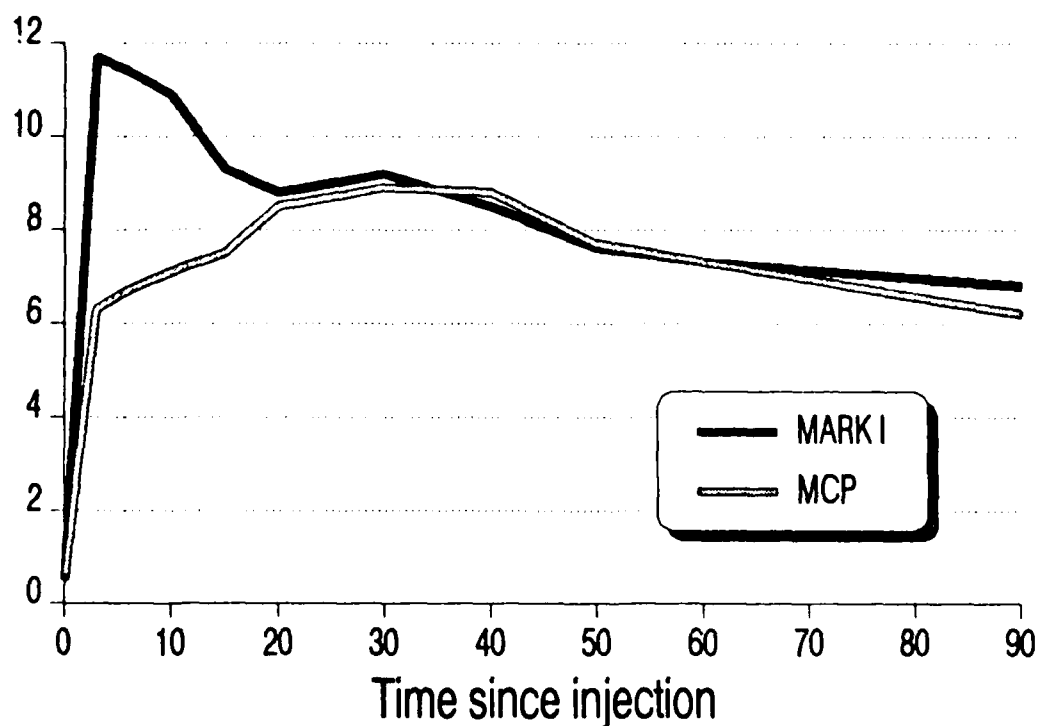


Figure 9. Serum atropine (RRA). Comparison of mean levels (ng/ml) in the first 90 minutes after administration.

Table 16. Serum Atropine (RRA) kinetics.

SUBJ	Tmax (mins)		Cmax (ng/ml)		AUC-90m (ng min/ml)		AUC-12h (ng min/ml)		Absorption (min)		Half Times Distrib't'n (min)		Elimination (min)	
	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP
1	30	3	9.3	15.6	793	1422	1900	5063					261	288
2	60	40	9.5	21.7	596	1127	2623	4509	33		7	127	127	282
3	3	10	19.0	11.6	928	657	2549	1682			14		98	
4	3	60	14.9	19.3	1652	1027	5804	1601					86	
5	30	3	15.6	11.2	1004	1072	1592	2347	2	15	40	15	30	106
6	3	3	32.4	21.7	1491	1302	2109	3799	3	1	13	15	197	275
7	6	15	15.6	16.9	1009	1726	2086	5916	4	49	17	35	78	153
8	6	30	19.6	9.7	1424	764	3823	1618	9		25		89	150
9	10	20	9.3	17.6	535	1440	971	4902	4		57	41	107	341
10	10	15	25.7	8.8	1782	704	6798	1163		5		17	258	101
11	40	90	11.5	11.5	1005	1027	3732	3849	11	26	33	26	202	254
12	3	90	10.8	6.4	498	661	991	1910						
13	10	20	11.5	8.1	1198	694	3526	3601					335	185
14	30	40	6.8	24.4	712	986	2347	2457				23	242	
15	3	50	18.3	10.3	1095	910	4179	2114					250	
16	6	10	13.5	6.1	1250	507	5387	1660	2	10	73	32	173	183
17	3	30	18.3	14.2	1275	586	5122	1090					340	151
18	6	50	9.3	11.4	765	814	1437	3930		5		30	77	238
19	10	3	16.9	11.5	1499	1259	5398	6622					319	335
20	3	30	22.7	10.0	1218	1045	2285	5536	9		11	106	140	230
MED	6	25	15.2	11.5	1052	1006	2586	3029	3	15	24	33	173	230
p	.022		NS		NS		NS		NS		NS		NS	

Note: Tmax = Time to reach first peak, Cmax = concentration @ peak, AUC-90m = area under curve from injection to 90 minutes, AUC-12h = area under curve from injection to 12 hours, medians computed for complete pairs only.

The median absorption half-times were 3 (range 2-11) minutes (MARK I) and 15 (range 1-49) minutes (MCP) (Table 16). This represented only 5 out of the 20 subjects where a meaningful absorption time could be computed. This inability to compute absorption times reflects the rapid rise to peak levels in atropine (RRA) and a true estimate of absorption incorporating all of these uncomputed values would be much shorter.

The median elimination half-times were 173 (30-340) minutes (MARK I) and 230 (86-341) minutes (MCP). This represented 15 of the 20 individuals and the values were not significantly different.

b. Comparison of RRA to RIA kinetics

Median peak levels were comparably measured by the two assays with: 12.8 ng/ml (RIA, MARK I), 15.2 ng/ml (RRA, MARK I) and 9.2 ng/ml (RIA, MCP), 11.5 ng/ml (RRA, MCP). However, when atropine levels were determined by RIA, the time to maximum was no longer significant but peak level now achieved significance (higher for the MARK I). In both assays, levels achieved by 10 minutes were significantly higher for the MARK I. Both assays were calibrated to atropine sulfate standard curves and all results were identically expressed in mass units of atropine sulfate equivalents.

The correlation between serum atropine by RRA and RIA was 0.65 and 0.42, with regression coefficients of 0.53 and 0.38, for the MARK I and MCP, respectively. These poor overall correlations and low regression coefficients are explained by the more rapid elimination of atropine RRA measureable activity from circulation although estimated elimination half times did not differ (Figure 10, Figure 11, Table 16, Table 18).

In comparison to atropine RRA, the longer time for the atropine RIA concentrations to reach the same peak levels made it possible to compute more of the individual estimates of absorption. For 11 subjects with complete pairs the medians were 3 (range 1-22) minutes (MARK I) and 8 (4-21) minutes (MCP) (Table 18). These were not different from the RRA values. Elimination half-times were not different and the medians were very similar to those obtained for the RRA except that the RIA results were consistent enough to allow all individual elimination coefficients to be computed. AUC-12 hours were comparable for the two assays but AUC-90 minutes medians were lower for the RIA ($p=0.024$, MARK I; $p=0.015$, MCP), presumably reflecting the longer rise to peak values. Atropine RIA distribution half-times were longer than RRA and also different between injectors, with MCP delivered atropine being more rapidly distributed.

No correlations between individual peak atropine measurements and peak physiologic responses were impressive and only one achieved significance (heart rate and atropine RIA (MARK I)) (Table 19).

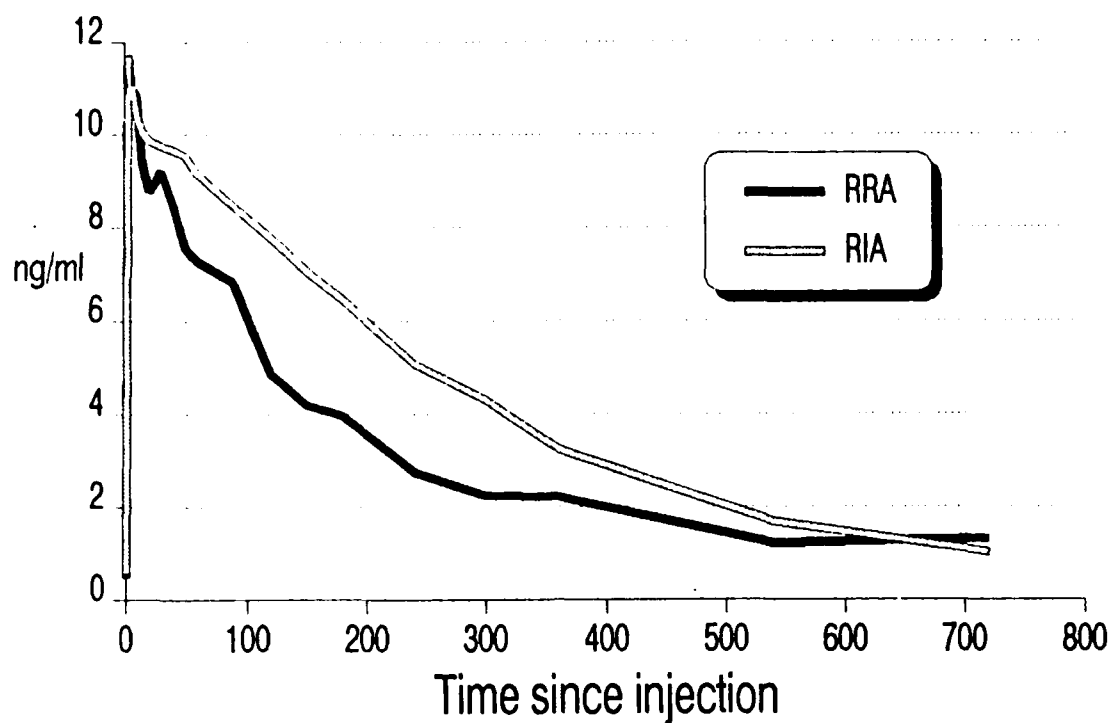


Figure 10. Serum atropine levels as measured by RRA and RIA, following administration by the MARK I autoinjector.

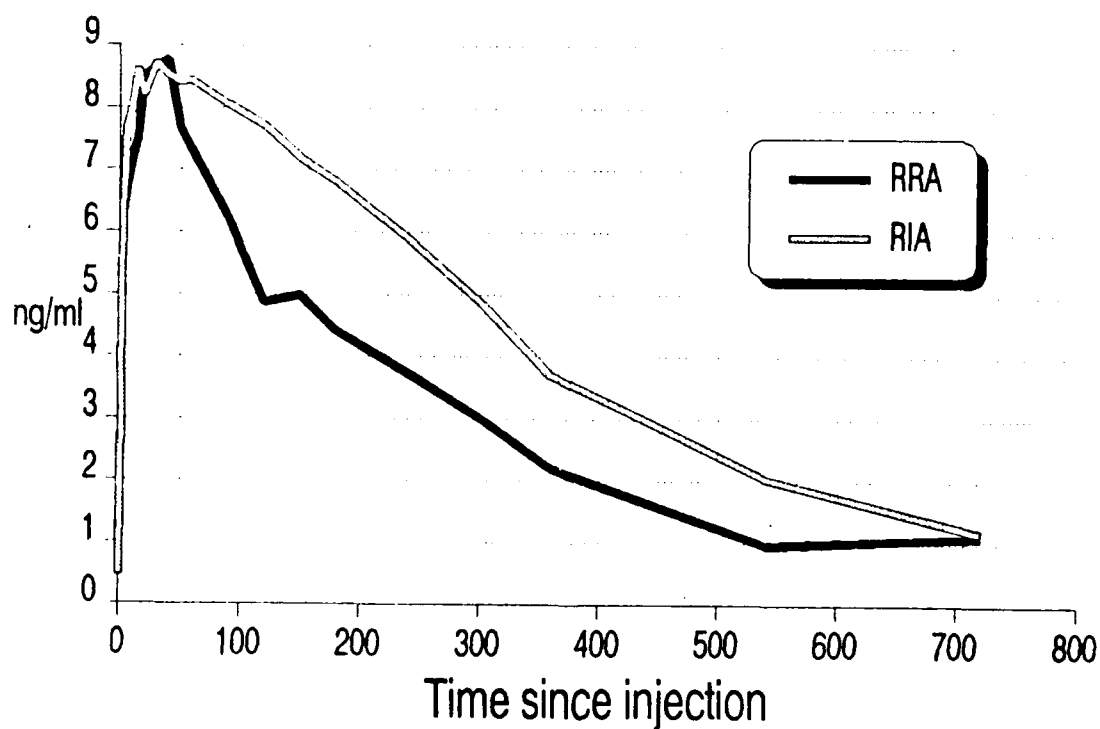


Figure 11. Serum atropine levels as measured by RRA and RIA, following administration by the MCP autoinjector.

Table 17. Mean serum atropine (RIA) concentrations (a.sulfate equiv) compared between injectors at each time period by paired t test.

Time (mins)	MARK-I		MCP		MEAN		t	prob
	ng/ml	SEM	ng/ml	SEM	DIFF	SEM		
Baseline	0.5	0.3	0.5	0.3	0.1	0.4	0.19	
3	10.8	1.1	6.3	1.0	4.4	1.0	4.70	0.001
6	11.1	0.8	7.7	0.9	3.5	0.9	4.05	0.001
10	10.4	0.9	8.1	0.9	2.3	0.8	3.01	0.008
15	10.0	0.9	9.0	1.0	1.1	0.9	1.17	
20	9.9	0.8	8.2	0.9	1.7	0.9	1.95	
30	9.8	0.8	8.7	0.9	1.0	1.0	1.01	
40	9.7	0.7	8.5	0.7	1.2	0.8	1.46	
50	9.5	0.7	8.4	0.7	1.1	0.8	1.35	
60	9.2	0.6	8.4	0.7	0.7	0.7	0.99	
90	8.5	0.6	8.1	0.5	0.4	0.6	0.64	
120	7.8	0.5	7.7	0.5	0.1	0.6	0.11	
150	7.1	0.5	7.2	0.5	-0.1	0.6	-0.24	
180	6.5	0.4	6.7	0.5	-0.2	0.5	-0.38	
240	5.1	0.4	5.9	0.5	-0.8	0.5	-1.69	
300	4.3	0.4	4.8	0.5	-0.5	0.5	-0.94	
360	3.2	0.3	3.7	0.4	-0.5	0.5	-0.99	
540	1.7	0.3	2.2	0.4	-0.5	0.4	-1.27	
720	1.0	0.3	1.2	0.4	-0.2	0.4	-0.58	

Serum Atropine Levels (RIA) (ng/ml)

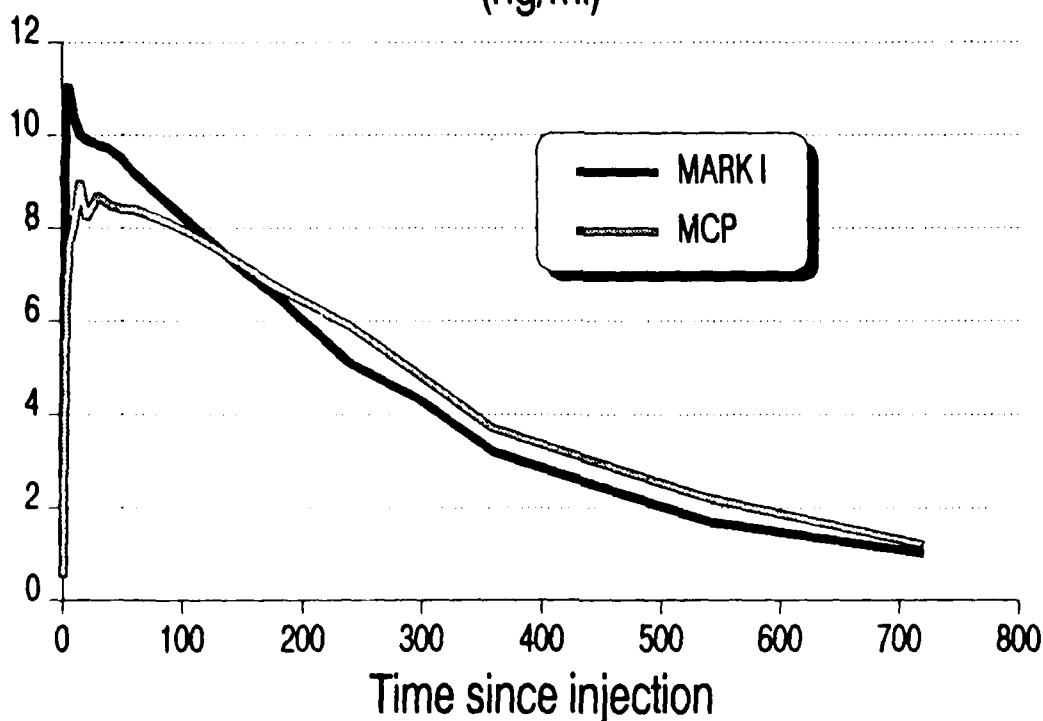


Table 18. Serum atropine (RIA) kinetics.

SUBJ	Tmax (mins)		Cmax (ng/ml)		AUC-90m (ng min/ml)		AUC-12h (ng min/ml)		Absorption (min)		Half Times Distrib't'n (min)		Elimination (min)	
	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP
1	40	6	12.4	9.3	967	750	3883	2637	9	4	57	7	167	196
2	6	10	9.4	3.4	495	248	1803	783	3	21	40	29	170	160
3	3	3	14.8	11.0	725	850	2434	5018	2		59		170	624
4	40	40	13.4	7.8	782	537	3469	3052	1	8	54	50	233	227
5	60	3	11.2	12.9	844	999	4124	3913	21		248		315	261
6	3	15	19.6	19.8	1313	1143	4035	3060	1		47	78	189	146
7	60	40	13.3	8.5	1060	624	5304	3172	22	7	147	56	410	200
8	15	15	12.9	16.0	690	1142	2901	4945	14	14	57	60	179	171
9	3	6	8.9	8.0	595	436	1576	1373		2		12	143	193
10	10	15	13.1	7.5	710	588	2058	2611		4		73	187	211
11	20	15	10.3	12.5	732	873	2898	3126	5	7	99	31	194	282
12	3	180	9.6	9.9	551	799	2056	4521	2	2	113	81	170	196
13	6	6	14.9	6.6	852	499	2396	2453	2	5	25	50	166	262
14	50	150	12.3	10.8	920	737	4443	5145		8	114	9	394	506
15	3	50	18.4	17.3	962	1312	2993	3605	15		74		234	186
16	6	60	10.0	10.8	746	867	2523	3825	3	11	41	22	184	194
17	3	3	10.2	7.0	446	405	1350	1849	2	10	54	27	126	180
18	6	20	16.2	9.1	1140	641	3980	2459	2		94	47	200	109
19	3	180	12.6	8.0	816	561	2479	3387	3	11	52	35	128	267
20	3	6	20.7	9.0	1292	647	3988	2828	7		55		230	346
med	6	15	12.8	9.2	799	692	2899	3093	3	8	55	41	186	198
p	NS		.0047		NS		NS		NS		.0273		NS	

Note: Tmax = Time to reach first peak, Cmax = concentration @ peak, AUC-90m = area under curve from injection to 90 minutes, AUC-12h = area under curve from injection to 12 hours, medians computed for complete pairs only.

Table 19. Correlation between amplitude of individual maximal changes in key physiological variables and corresponding peak serum atropine measurements.

	MARK I		MCP	
	RRA	RIA	RRA	RIA
Maximum heart rate	-0.093	0.490 **	-0.247	0.379
Maximum change in heart rate	-0.125	0.370	-0.082	0.391
Minimum sal secretion	0.117	-0.149	0.024	-0.118
Maximum change in pupil area	-0.078	0.210	-0.304	0.032

**significance, $p < 0.05$

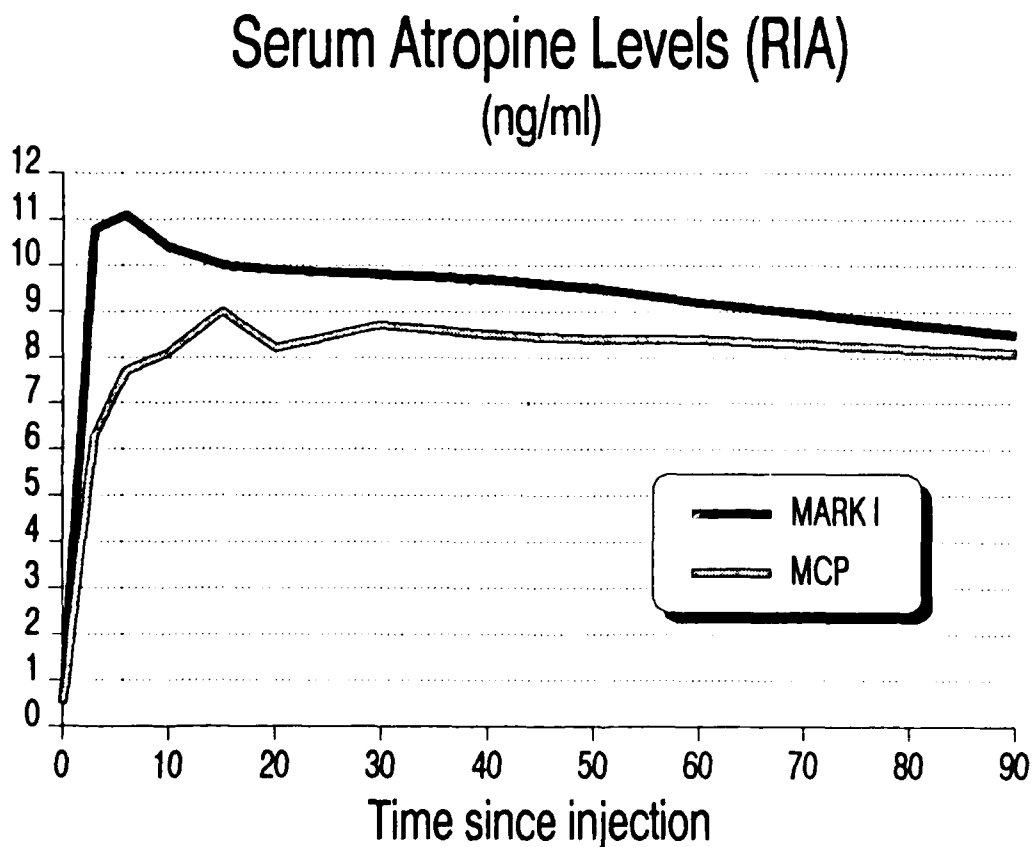


Figure 12. Serum atropine (RIA). Comparison of mean levels (ng/ml) in the first 90 minutes after administration.

Table 20. Mean blood pralidoxime chloride concentrations compared between injectors at each time period by paired t test.

Time (mins)	MARK-I		MCP		MEAN		t	prob
	ug/ml	SEM	ug/ml	SEM	DIFF	SEM		
Baseline	0.0		0.0					
3	1.4	0.2	1.6	0.2	-0.2	0.3	-0.77	
6	2.3	0.3	2.9	0.3	-0.6	0.3	-1.97	
10	2.9	0.3	3.7	0.3	-0.9	0.3	-3.22	0.005
15	3.2	0.3	3.6	0.3	-0.4	0.3	-1.50	
20	3.0	0.2	3.5	0.2	-0.5	0.3	-2.03	0.056
30	3.2	0.2	3.3	0.2	-0.2	0.2	-0.96	
40	2.8	0.1	3.1	0.2	-0.3	0.2	-1.61	
50	2.7	0.1	2.8	0.2	-0.1	0.1	-0.41	
60	2.5	0.1	2.9	0.2	-0.3	0.1	-2.49	0.022
90	2.5	0.2	2.5	0.2	0.0	0.3	0.04	
120	2.4	0.1	2.1	0.1	0.3	0.2	1.82	
150	2.0	0.1	1.7	0.1	0.3	0.2	2.05	0.054
180	1.9	0.1	1.7	0.2	0.2	0.2	1.35	
240	1.5	0.1	1.4	0.2	0.2	0.2	0.76	
300	1.2	0.2	1.0	0.1	-0.2	0.2	1.06	
360	1.1	0.2	0.8	0.1	0.3	0.2	1.68	
540	0.9	0.1	0.4	0.8	0.6	0.2	3.15	0.006
720	0.6	0.1	0.3	0.1	0.3	0.1	2.17	0.046

2 PAM Cl ug/ml

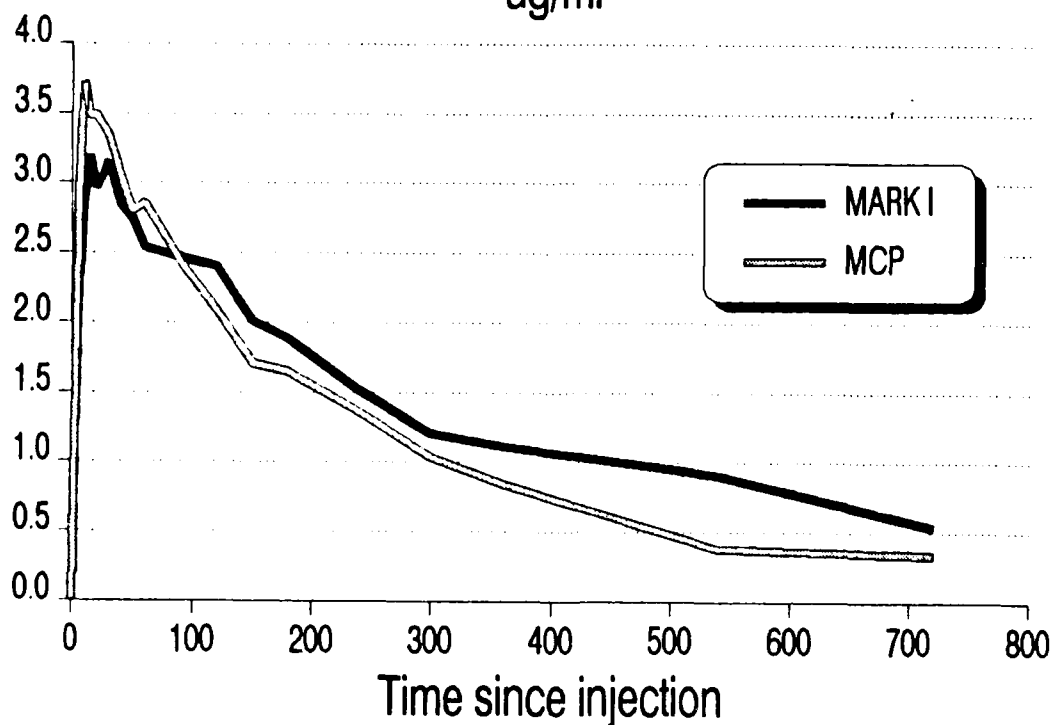


Table 21. 2-PAM kinetics.

SUBJ	Tmax (mins)		Cmax (mcg/ml)		AUC-90m (mcg min/ml)		AUC-12h (mcg min/ml)		Absorption (min)		Half Times Distrib't'n (min)		Elimination (min)	
	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP	MI	MCP
1	10	15	3.0	4.1	310	361	1748	1524		1	25	18	187	109
2	20	10	2.2	2.3	256	255	1585	1367					693	459
3	6	20	2.7	4.1	272	357	1925	1928		5		96		118
4	30	40	4.1	4.3	347	407	1851	2088	5	82	231	65	230	845
5	15	15	4.7	4.7	380	422	1828	1868					154	174
6	15	6	5.0	6.6	468	473	2334	2044	34	10	32	83	209	491
7	50	20	2.5	3.9	346	328	1950	1613			36	29	62	181
8	10	20	4.8	4.6	366	465	2043	2353	5		237		233	262
9	30	10	3.6	4.0	344	324	1487	1414	5	1	88	78	327	88
10	10	10	5.2	4.8	373	445	1995	1971	11	3	90	57	305	149
11	20	15	3.8	4.7	341	457	1847	1896	7	7	28	49	203	636
12	30	6	3.5	2.1	323	333	1729	1927	4		67		139	
13	40	6	2.2	3.5	292	285	1789	1526		11		12	231	204
14	40	30	3.5	5.4	318	425	1823	2138	21		8	335	141	506
15	30	6	3.6	5.1	426	447	2058	2039	13	1	32	33	392	499
16	15	20	4.2	4.4	361	423	1493	2046	2		70	59	94	70
17	10	60	2.8	2.5	290	327	1750	1587	9		33	15	213	85
18	40	90	3.6	5.3	375	437	2292	1525	83	29	100	37	462	78
19	15	10	5.2	3.9	311	365	1648	1673	3	7	52	7	278	153
20	20	10	4.1	6.6	432	354	2105	1310		9	27	13	92	143
med	20	15	3.62	4.35	345	386	1837	1882	11	7	35	43	222	178
p			NS		NS		NS		NS		NS		NS	

Note: Tmax = Time to reach first peak, Cmax = concentration @ peak, AUC-90m = area under curve from injection to 90 minutes, AUC-12h = area under curve from injection to 12 hours, medians computed for complete pairs only.

c. Blood pralidoxime chloride

Blood concentrations of 2-PAM are shown in Table 20. There was no significant difference between median time to peak or for peak concentrations. Peak values were: 3.6 ug/ml (approx 6.4 ug/ml serum)(MARK I) and 4.3 ug/ml (approx 7.6 ug/ml serum)(MCP)(Table 21). The concentration of 2-PAM achieved by the MCP was significantly higher at the 10 minute interval and tended to be higher through the first hour after injection (Table 20, Figure 12). AUC-90 minutes, AUC-12 hours, and estimable absorption and elimination half-times were not significantly different between injectors (Table 21).

3. Effects of eye color.

Eye color was a significant covariate in the effect of atropine on heart rate. In a repeated measures analysis, there were significant interactions between eye color (blue, hazel, brown) and time and between injector and time. There were no significant interactions involving injector and eye color. Individuals with the most pigmented irides showed a greater heart rate response to atropine than those with less pigmented eyes. Brown and blue eyes were at the extremes and hazel-eyed subjects were somewhat intermediate (Table 22).

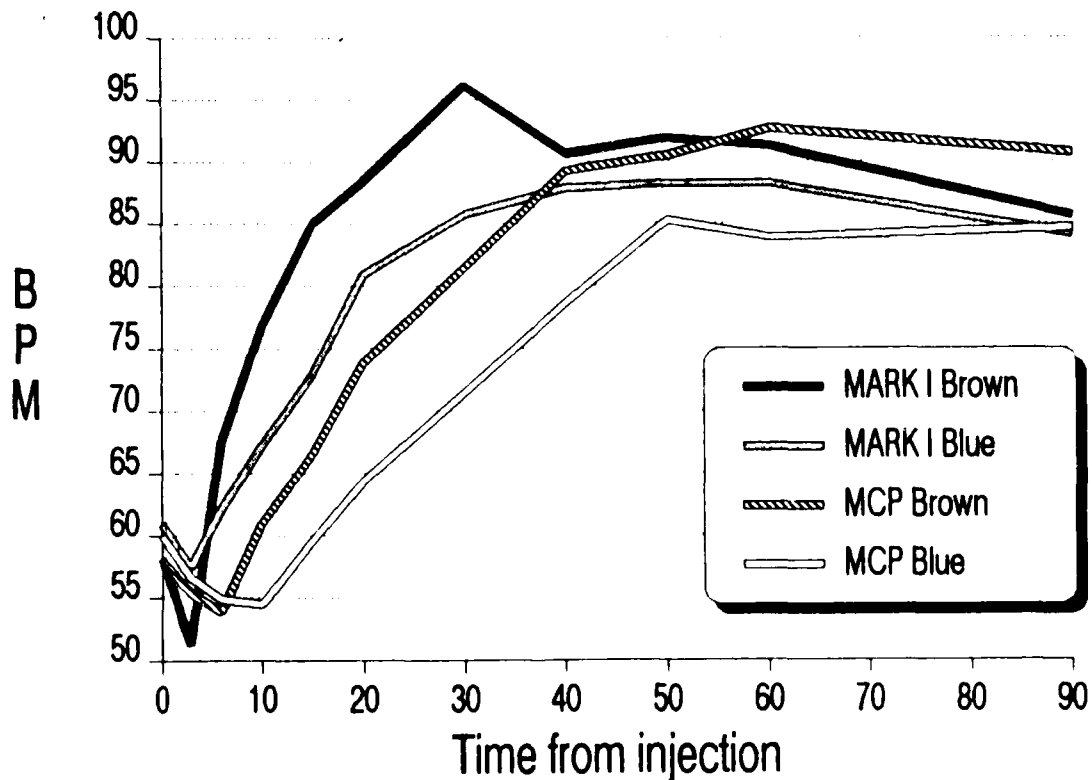


Figure 13. Mean heart rates of blue and brown eyed subjects divided according to injector.

Table 22. Change in Heart Rate from baseline. Values are compared between blue- and brown-eyed subjects by an unpaired t-test.

Time (mins)	Blue (bpm)	SEM	Brown (bpm)	SEM	Hazel (bpm)	SEM	Blue vs Brown t	p value
0	0.0	-----	0.0	-----	0.0	-----		
3	-3.4	1.9	-4.8	1.2	-5.7	1.7	0.63	
6	-2.1	1.7	2.5	2.6	-4.3	3.9	-1.47	
10	0.4	2.9	10.7	3.0	2.3	3.6	-2.48	0.019
15	5.8	2.9	16.1	3.1	8.8	5.1	-2.39	0.024
20	12.1	3.6	21.5	3.2	14.8	5.3	-1.95	
30	18.1	3.2	29.9	3.4	19.9	5.2	-2.54	0.017
40	22.8	2.7	31.8	2.4	23.8	4.3	-2.52	0.017
50	26.3	2.4	33.0	2.2	23.8	3.9	-2.09	0.046
60	25.6	2.2	33.8	2.4	21.9	3.5	-2.49	0.019
90	23.9	2.2	29.4	2.8	18.9	3.2	-1.57	
120	16.9	2.8	24.1	2.5	17.8	1.8	-1.93	
150	12.5	2.5	22.4	2.5	12.6	2.4	-2.79	0.009
180	5.6	2.5	13.8	2.6	11.3	2.2	-2.23	0.034
240	2.3	2.4	9.5	2.3	3.8	1.6	-2.17	0.038
300	2.3	2.8	1.9	1.6	1.4	2.6	0.14	
360	-1.5	2.5	2.9	1.7	0.4	2.9	-1.44	
540	-4.4	2.1	3.2	1.9	-3.7	2.7	-2.67	0.012
720	-3.4	2.2	2.4	2.3	0.6	1.8	-1.79	

Note: Means (\pm SEM) are based on 2 observations per subject, with subjects n=8 (blue eyes), 8 (brown eyes), 4 (hazel eyes).

Heart Rate by Eye Color

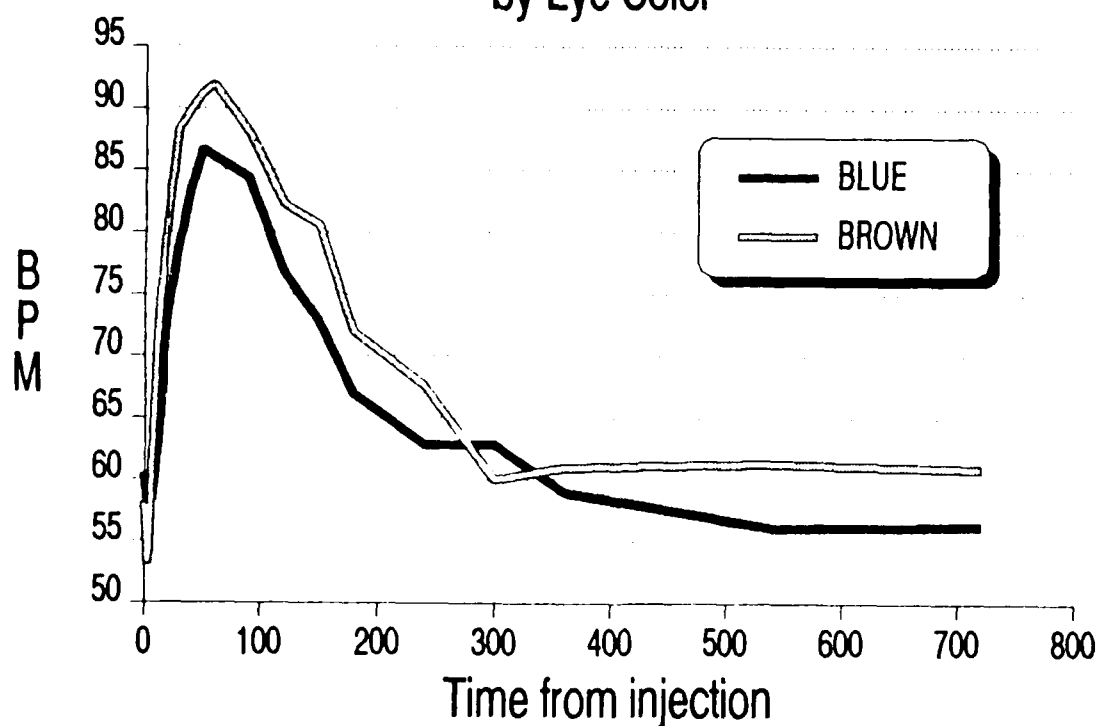


Table 23. Change in Heart Rate from baseline. Values are compared between blue- and brown-eyed subjects by an unpaired t-test.

MARK I

Time (mins)	Blue (n=8)		Brown (n=8)		Hazel (n=4)		Blue vs Brown	
	(bpm)	SEM	(bpm)	SEM	(bpm)	SEM	t	p value
0	0.0	-----	0.0	-----	0.0	-----		
3	-3.5	1.7	-6.9	1.7	-5.4	1.9	1.40	
6	1.0	2.8	9.2	3.6	2.8	4.8	-1.79	
10	6.3	4.7	18.6	3.5	10.6	3.9	-2.12	
15	12.0	12.8	24.7	3.8	19.1	7.0	-2.12	
20	19.9	5.1	28.2	4.5	24.1	8.2	-1.20	
30	24.8	3.1	37.3	4.1	27.6	8.2	-2.46	0.029
40	26.9	3.1	32.5	4.0	27.8	8.0	-1.09	
50	27.3	3.2	33.7	4.0	24.8	8.0	-1.26	
60	27.3	3.6	33.1	4.7	22.1	7.3	-0.98	
90	23.2	4.0	27.5	4.5	17.1	6.4	-0.72	
120	15.7	5.3	20.4	4.5	15.8	2.9	-0.67	
150	11.4	4.3	22.1	4.4	11.8	4.0	-1.73	
180	2.0	3.4	11.0	5.0	11.8	4.3	-1.43	
240	1.9	4.5	7.6	3.4	2.8	2.1	-1.02	
300	1.5	5.0	-0.7	2.7	-1.4	4.4	0.38	
360	-1.6	4.7	2.7	2.4	-1.2	5.1	-0.82	
540	-7.1	3.7	2.0	2.9	-1.7	1.2	-1.93	
720	-3.8	3.9	2.4	4.1	0.3	2.2	-1.06	

MCP

Time (mins)	Blue (n=8)		Brown (n=8)		Hazel (n=4)		Blue vs Brown	
	(bpm)	SEM	(bpm)	SEM	(bpm)	SEM	t	p value
0	0.0	-----	0.0	-----	0.0	-----		
3	-3.4	3.4	-2.8	1.6	-6.0	3.0	-0.17	
6	-5.2	1.4	-4.3	1.5	-11.5	3.8	-0.43	
10	-5.5	2.2	2.9	2.8	-6.0	0.9	-2.32	0.036
15	-0.4	2.3	8.5	2.9	-1.5	1.5	-2.40	0.031
20	4.4	3.5	15.7	3.7	5.6	2.6	-2.24	0.042
30	11.5	4.5	23.4	4.1	12.3	4.7	-1.95	
40	18.6	4.0	31.1	2.9	19.8	3.7	-2.54	0.024
50	25.2	3.7	32.4	2.2	22.8	2.1	-1.64	
60	23.9	2.8	34.5	1.7	21.8	2.3	-3.21	0.006
90	24.6	2.2	31.7	3.2	20.8	1.9	-1.84	
120	17.9	2.7	27.9	1.9	19.8	2.2	-3.02	0.009
150	13.8	2.6	22.7	2.6	13.3	3.3	-2.40	0.032
180	8.7	3.5	16.6	1.6	10.8	1.8	-2.03	
240	2.7	2.0	11.4	3.2	4.8	2.7	-2.29	0.038
300	3.1	3.0	4.4	1.2	4.3	2.7	-0.39	
360	-1.4	2.2	3.0	2.6	2.1	3.5	-1.30	
540	-1.7	1.6	4.4	2.6	-5.7	5.4	-1.96	
720	-3.0	2.8	2.4	2.0	1.0	3.5	-1.53	

These differences also divided along ethnic lines with all of the eight blue eyed subjects being caucasian and only 2 of the brown-eyed subjects were similarly classified (Table 2). There was no significant difference between blue and brown eyed subjects in terms of body weight or lean body mass. There was no difference between blue and brown eyed subjects in terms of serum atropine (RRA) levels achieved.

The effect on heart rate by eye color extremes (blue and brown) and by injector is shown in Figure 13. Brown-eyed subjects had the same level of heart rate response following injection with the MCP as the level achieved by blue eyed individuals following injection with the MARK I, indicating that eye color was a variable affecting the heart rate response in a magnitude comparable to the difference observed between injectors.

4. Other effects relative to injection.

a. Serum rise in CPK

CPK was significantly elevated following injection by either injector with no differences between injectors and with a linear rise over time through 6 hours post-injection. At 6 hours, mean levels were increased by approximately 150 U/l (Figure 14). This was very similar to the previously reported CPK rise induced by 2-PAM administered by manual intramuscular injection (Sidell, Culver & Kiminskis, 1974).

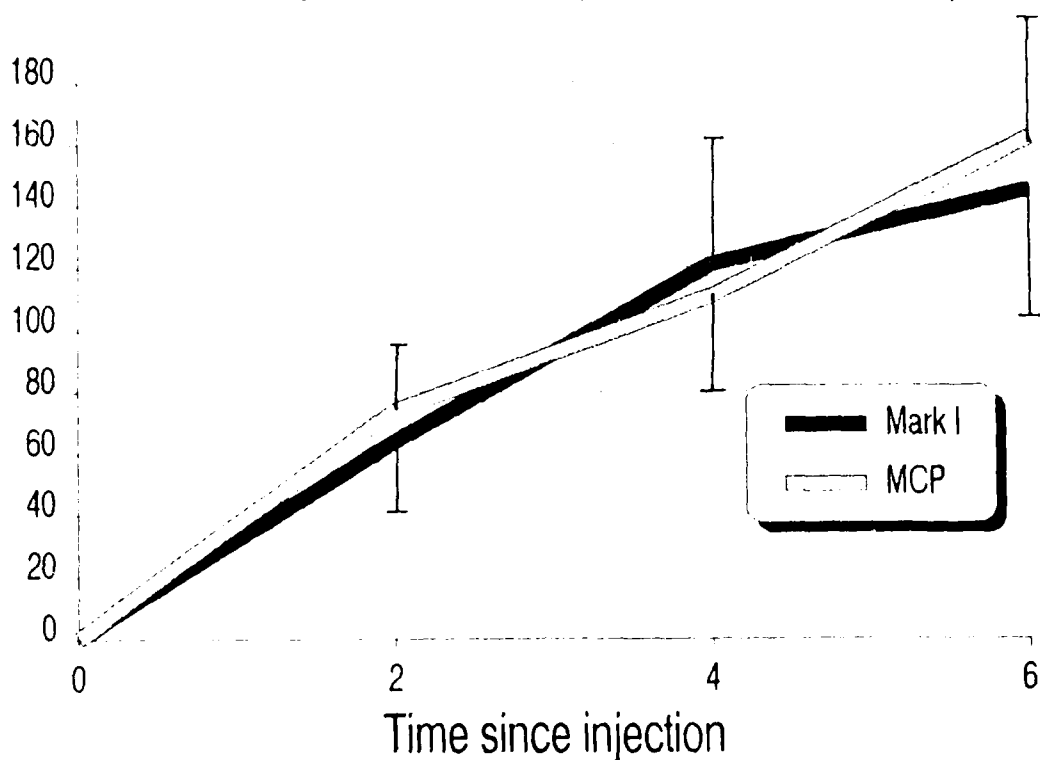


Figure 14. Change in serum CPK (U/ml) compared between injectors. Vertical bars represent SEM.



Figure 15. Typical appearance of needle punctures following injection of a leg with the MARK I device. In this case the atropen (puncture at left) also created a slightly raised and blanched welt (subject no. 10). This occurred in three of the twenty subjects.

b. Pain

Pain was reported by most subjects in this study following injection with either device; however, the pain was not directly attributed to the injection. In most cases, there was no sensation from the injection itself but pain began seconds to minutes after the needle was removed. This was most commonly described as a "charley-horse" sensation or the feeling of intense cramping in the upper leg. This sensation usually lasted from 2 to 4 hours. In other studies, pain has been specifically attributed to the 2-PAM component (Haegerstrom-Portnoy et.al. 1987; Barkman, Edgren & Sundwall, 1963).

c. Dermal reactions

Three (Nos. 7, 10, 20) out of twenty subjects injected with each injector developed a welt, approximately 1 to 1-1/2 inches in diameter, noticeable almost immediately after injection with the atropen cartridge of the MARK I device (Figure 15). This was slightly discolored (blanched) in two of the three cases and was not associated with any other symptoms or with any differences in serum atropine levels compared to the remainder of the group. The welts disappeared within approximately 2 hours.

d. Mechanical problems

In one instance (subject No. 15), the needle of the combopen portion of the MARK I injected and was withdrawn with obvious resistance by the investigator. This was attributed to a hook formed when the bevel of the needle was bent back toward the needle, away from the bore. The needle had fired off-center through the rubber end cap of the injector and appeared to have glanced off of the plastic collar. This is thought to have produced the defect. The subject stated that he did not experience any pain and that he had not felt the injection at all. There was no evidence that the needle reached the femur in this or any other subjects.

In many cases, the combopen portion of the MARK I devices could not be activated by pushing into the subject leg and instead had to be struck against the leg in order to fire. The same degree of activation force was not required for either the atropen portion of the MARK I or for the MCP.

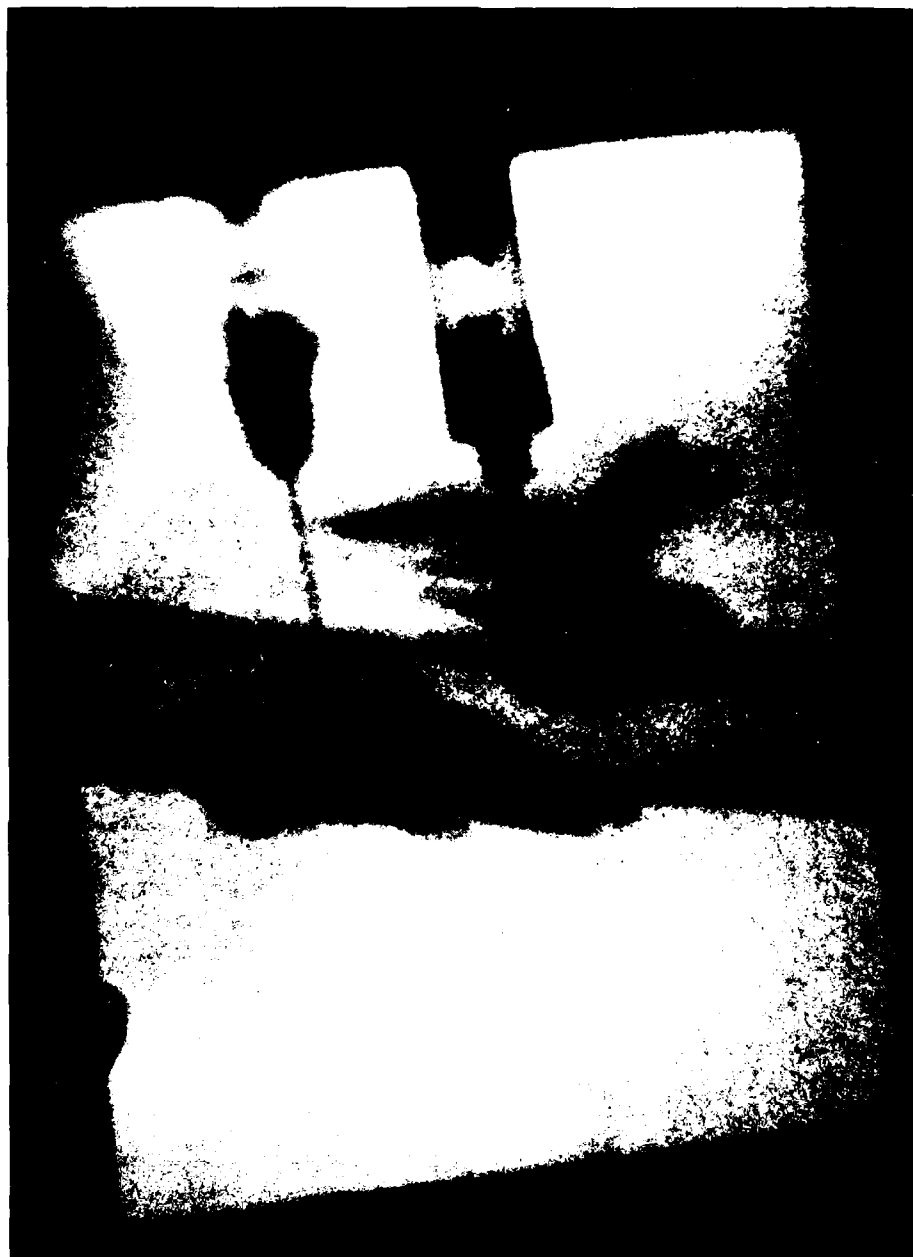


Figure 16. Delivery of radioopaque material into a dog leg by the MARK II, a device consisting of the atropen and combopen cartridges but arranged to inject simultaneously (from a contract study by Survival Technology, Inc.). This illustrates differences between the two injectors in the extent of intramuscular dispersion of injector contents. The atropen is pictured at right.

DISCUSSION

Serum atropine, heart rate, and salivary secretion changed more rapidly following injection with the MARK I while 2-PAM in the blood tended to increase more rapidly after injection with the MCP. All of these changes were significant at the sampling interval 10 minutes after injection. In order of significant change from baseline, serum atropine and blood 2-PAM levels changed first (3 mins), followed by salivary secretion (6 mins), heart rate increase (10-20 mins), and change in pupil area (30 mins). The initial decrease in heart rate and the decrease in salivary secretion were both already evident at the first (3 minute) interval confirming that these are relatively rapid and useful pharmacodynamic markers of atropine action.

The differences in atropine delivery may be related to the design of the injectors. The atropen begins delivering drug as the needle is being moved forward while the MCP (and the combopen) does not begin drug delivery until the needle is fully extended. We confirmed this with a pedestrian technique, firing each injector through a stack of 0.5 cm plastic bubbles. The first bubble and each subsequent bubble along the course of the atropen needle contained fluid. The MCP injector filled and ruptured only the last bubble reached by the extended needle. The effect of this difference has been demonstrated by the manufacturer in studies comparing the tissue distribution of radioopaque dye (Figure 16). The atropen clearly has a broader field of dispersion and this alone would be expected to enhance absorption. Unfortunately, 2-PAM does not store well in contact with metal and delivery from a device such as the atropen with a metal jacketed drug container and needle residing in the solution is currently impractical (May & Kondritzer, 1965).

The action of the atropen may explain the welts seen in three out of twenty subjects injected with this device. These welts may have been dermal infiltrations produced by early delivery of the drug and the blanching effect is consistent with this explanation. The possibility of such an action in the skin raises a question about the applicability of the results of this study to the field environment. Soldiers would usually be expected to inject through one or more layers of clothing (perhaps including a relatively thick chemical protective overgarment) and, delivered by the atropen, some of the atropine might be delivered into the clothing before injecting the tissue. When fired through a single thickness of the chemical protective suit (approximately 2 mm), and before withdrawal, the atropen produces a wet ring and the combopen does not. If this is a consistent phenomenon, this will reduce or reverse the differences between injectors noted in this study.

Although the 2-PAM was administered by injectors with similar actions, different volumes of fluid were delivered (2.0 mls from the combopen and 2.7 mls from the MCP). The larger volume from the MCP may have increased the absorption

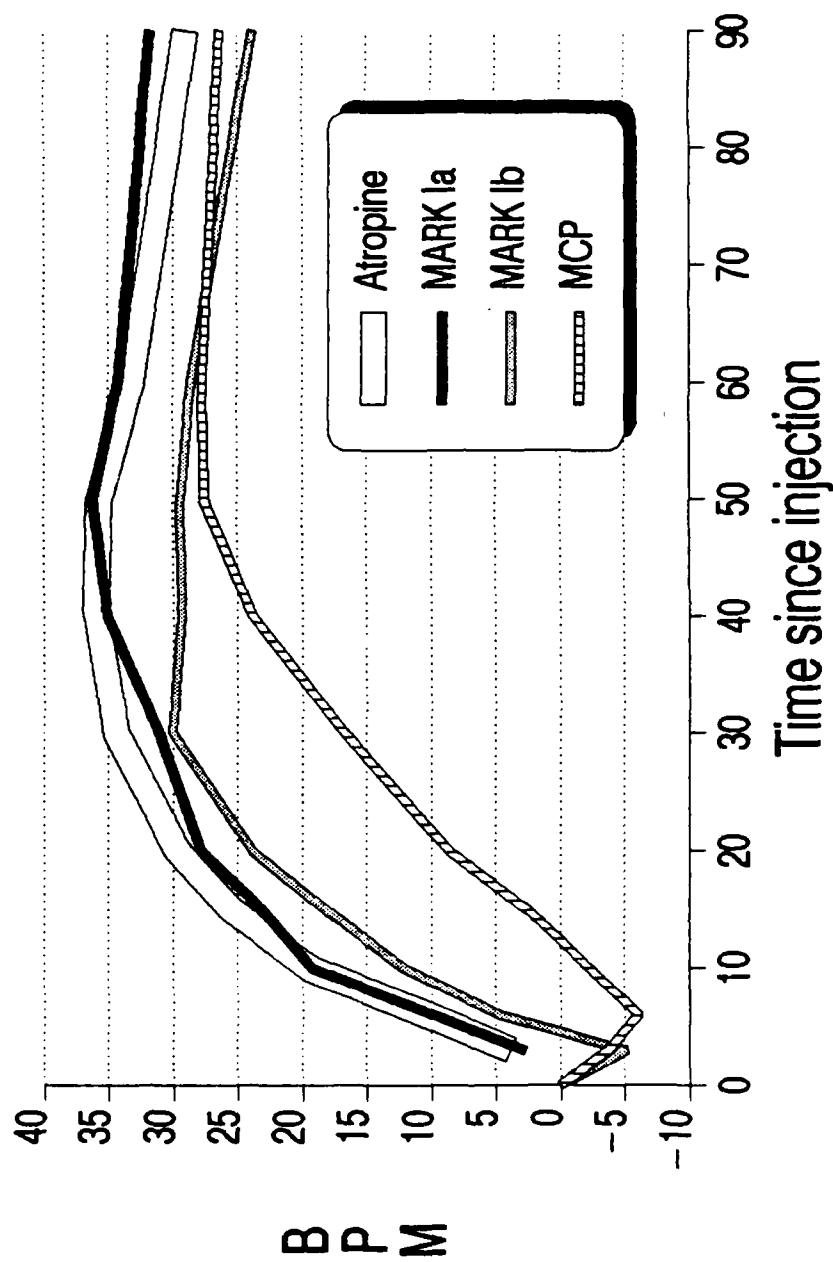


Figure 17. Mean change in heart rate compared between the current study and Riley & Perkal (1985). Atropine and MARK Ia curves were obtained in the previous study following injection of men by atropen and MARK I. MARK Ib and MCP curves show the data collected in the current study.

of 2-PAM by delivery of the same dose of 2-PAM in a larger bolus with a necessarily larger area of distribution in the muscle. 2-PAM absorption might also be improved by the same mechanism which Sidell postulated for delayed atropine absorption, with a reduction in osmolality of the 2-PAM solution when diluted by the atropine solution.

The observed heart rate responses and the atropine (RIA) levels following injection with the MARK I in this study match well to the results of a previous study with the MARK I (Figure 17 and Table 24). On that basis, comparison to the results of the previous study provides further confirmation that delivery of atropine by the MCP was not as rapid as from the MARK I and it was also slower than delivery achieved by the atropine alone.

Certain differences in heart rate responsiveness to atropine have been previously attributed to the degree of pigmentation, either by ethnic origin or eye color (Paskind, 1921; Fry & Hall-Parker, 1974). In this study those observations were confirmed with the finding that eye color may be a significant covariate in the heart rate response. The brown-eyed individuals also represented all of the more pigmented ethnic groups identified and so the effect of total body melanin and iris pigmentation cannot be distinguished. Nevertheless, these differences were at least as large as the difference in response between injectors. Although this does not alter the basic interpretation of the results in this study (because of the

Table 24. Comparisons of medians between the present study and Riley & Perkal (1985) using the Kruskal-Wallis test. There were no significant differences between any of the first three columns; significance is indicated for differences between injectors in the current study.

Parameter	--Previous study--		--Current study--		p val
	Atropen	MARK I	MARK I	MCP	
H E A R T R A T E					
Baseline	61.6	63.4	61.0	60.5	0.001
HR-10 min	76.3	80.4	76.0	60.5	
HR-max	95.7	102.3	96.0	89.5	
Time-max	40	50	40	50	0.001
dHR-10 min	11.8	15.8	14.2	-3.9	
dHR-max	30.7	36.8	33.9	31.7	
S E R U M A T R O P I N E (R I A)					
C-10 min	10.0	9.9	10.0	7.3	0.042
C-max	10.4	10.8	12.8	9.2	0.005
Time-max	10	10	6	15	
AUC-12 h	3044	3082	2899	3093	
Half time	185	180	209	246	

Note: All values shown are medians; times are computed for both studies only for intervals available in the current study.

crossover design), it underscores the importance of controlling for this factor. For example, when compared to heart rate responses following injection by the MARK I in the previous study (Riley & Perkal, 1985), the lines superimpose if blue eyed individuals are excluded from the current data. In the previous study, the group is thought to have consisted entirely of brown-eyed individuals. In that study, there was no significant bradycardic phase observed for the mean heart rates and this follows previous observations (Paskind, 1921) where no bradycardic phase occurred in 20 black subjects while a substantial bradycardia occurred in the 20 white subjects. The rate at which atropine reaches target tissues appears to determine the appearance of a bradycardia, with low rates achieving a sustained bradycardia (Lonnerholm & Widerlov, 1975). It is revealing that the more rapid absorption of atropine following injection with the MARK I tended to obscure differences between subject eye color groups while there was a marked difference in the duration of bradycardia between brown- and blue-eyed subjects with the slower atropine absorption following MCP administration.

The differences between the two atropine assay methods are consistent with the thinking that the RRA-measured atropine (primarily, 1-hyoscyamine) is preferentially removed from circulation. The RRA measures only the amount of drug or metabolite possessing significant affinity for the muscarinic receptor. Peak levels were closely matched between the two assays but subsequent bioassayable (RRA) levels rapidly diminished to approximately 60% of the immunologically reactive (RIA) levels. Aaltonen et.al. (1984) have reported a similar pattern in their intravenous atropine sulfate levels drawn from anesthetized patients, although we did not observe the 3-4 fold difference in AUC between assays which they found. They also calculated a shorter elimination half-time for the RRA-measured atropine while the volume of distribution was substantially larger. They speculated that this may be due to a phenomenon of preferential tissue uptake of the 1-hyoscyamine, similar to the example of propranolol isomers (Kawashima, Levy & Spector, 1976). Calculation of peripheral compartment levels and comparison to effect might clarify what is being measured in each assay but goes beyond the scope of this study.

Estimates of the relation between serum atropine levels and atropine effect are available from this study. The median times to serum atropine peak, ranging from 6-25 minutes, were followed by maximum heart rates and minimum salivary secretory rates at 40-50 minutes. This suggests a delay of at least 30 minutes for serum atropine to reach target tissue receptors, interpreted on the basis of the access-limited model of atropine effect (Thron & Waud, 1967). This compares to a 7-8 minute delay in the intravenous atropine sulfate study by Hinderling et.al. (1985). Hinderling et.al. (1985) have demonstrated that physiological effects (heart rate and salivary flow rate)

correlate well with the amounts of drug estimated for the peripheral compartment although these relationships, in a semilog plot, are not simply linear. Their studies predict a 90% of maximal heart rate effect at 3.1 ± 1.1 (SD) mg atropine (base) dose. We were well below such a saturation point with the 2 mg dose.

A single autoinjector dose of atropine in a healthy young man would produce heart rate responses close to the 95 bpm recommended by current Army medical doctrine as a dosing endpoint in a nerve agent casualty. Nevertheless, using this heart rate as an endpoint, 8 out of the 20 subjects injected with the MARK I would have taken a second dose if they thought they had been poisoned, even in the absence of an opposing nerve agent.

Our median elimination half-times ranged from 2.9 to 3.8 hours. These values are in the range of other averages reported for atropine or atropine sulfate: 2.1 hours (Wurzbarger et.al. 1977; Virtanen, Kanto & Iisalo, 1980), 2.2 hours (Hinderling, Gundert-Remy & Schmidlin, 1985), 3.0 hours (Riley & Perkal, 1985), 3.4 hours (Smallridge et.al. 1987), 4.1 hours (Harrison et.al. 1986; Adams et.al. 1982), and 3.7-4.3 hours (Aaltonen et.al. 1984).

This study indicates that there are differences between the two injection devices in terms of the circulating drug levels and pharmacodynamic endpoints achieved. These differences are largely confined to the first 40 minutes following injection. It can be speculated that the reasons for the differences include the action of the injectors (length of the injection trail) and probably also relate to differences in concentration of the solutions, as originally documented by Sidell with manual injection. This latter effect may work in opposite directions for the two drugs when combined: if the increased osmolality of the atropine solution impedes atropine absorption, dilution of the 2-PAM might increase 2-PAM absorption, as seen in this study.

It should again be cautioned that the differences noted in this study may not persist in a field environment. Injection through clothing, especially the relatively thick chemical protective suit, results in the loss of some atropine from the atropen device. Absorption will be enhanced if soldiers massage the injection site; this was specifically prohibited in our study. Vigorous activity would also be expected to enhance absorption, although it apparently does not alter the relative absorption of mixtures of atropine and pralidoxime methylsulfate compared to absorption when the components are individually administered (Martin, 1973). Finally, it must be noted that when these drugs are administered to oppose the effects of actual agent exposure, the kinetics will be substantially different (Green, Reid & Kaminskis, 1985).

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[illegible]

Appendix Table 2. Standard clinical serum biochemical parameters for individual subjects. Values are shown for blood urea nitrogen (BUN), creatinine (creat), total bilirubin (bili), alkaline phosphatase (alk phos), lactate dehydrogenase (LDH-L), glutamic oxaloacetic transaminase (SGOT) and, glutamic pyruvic transaminase (SGPT).

No.	BUN mg/dl	creat mg/dl	bili mg/dl	alk phos U/l	LDH-L U/l	SGOT U/l	SGPT U/l
1	17	0.4		83	108	20	14
2	13	0.6	0.3	72	132	18	19
3	15	0.8	0.5	60	151	16	21
4	15	1.2	0.2	64	154	18	18
5	15	0.7	0.4	85	212	51	66
6	19	1.0	0.3	73	237	31	16
7	15	0.8	0.3	68	159	18	22
8	10	0.7	0.2	54	150	17	21
9	13	0.8	0.5	68	146	18	13
10	10	0.7	0.3	129	167	20	24
11	24	1.0		80	160	16	13
12	21	1.1	0.6	77	110	15	11
13	14	1.0	0.4	56	109	13	9
14	12	0.8	1.3*	70	129	13	13
15	14	1.1	0.5	89	126	18	13
16	18	0.7	0.5	92	175	19	21
17	13	1.0	0.3	59	113	17	20
18	14	0.8	0.4	56	149	26	19
19	15	0.8	0.5	59	208	13	14
20	10	0.7	0.4	74	161	37	23
Laboratory normal ranges							
Lower	7	0.6	0.1	41	88	7	2
Upper	21	1.6	0.9	133	230	39	54

*Gilbert's hyperbilirubinemia

Appendix Table 3. Unfired injector weights (n=20 injectors selected at random from samples of 50 each). Injectors used in this study were required to fall within the 95% confidence interval (*) in order to minimize variance and to prevent any gross errors in dosing.

(grams)	Mean (SEM)	95% confidence interval

MARK I		
with holder	59.28 \pm 0.04	59.19 - 59.36
atropen	17.17 \pm 0.02	17.12 - 17.21 *
combopen	32.95 \pm 0.02	32.91 - 32.99 *
MCP		
with cap	32.56 \pm 0.01	32.54 - 32.59
without cap	31.53 \pm 0.01	31.51 - 31.55 *

Appendix Table 4. Summary of food composition from 10 lunches and 10 suppers served to the experimental subjects*. In general, the only sampling periods which could be affected by these meals were the +9.0 hr (after lunch) and +12.0 hr (after lunch & dinner) points.

Component	Lunches (\pm SD)	Suppers (\pm SD)
Total calories (kcal)	1382 \pm 251	1386 \pm 186
Total weight (gms)	1483 \pm 149	1293 \pm 134
Protein (gms)	78.4 \pm 16.4	64.0 \pm 4.7
Fat (gms)	49.7 \pm 17.2	58.5 \pm 18.8
- saturated (gms)	15.0 \pm 7.4	23.1 \pm 10.0
- oleic acid (gms)	13.2 \pm 3.4	19.1 \pm 6.1
- linoleic acid (gms)	7.1 \pm 2.3	8.7 \pm 2.9
- polyunsat:sat ratio	0.5 \pm 0.3	0.4 \pm 0.2
Carbohydrates (gms)	162.6 \pm 19.7	161.3 \pm 21.6
% protein	22.8 \pm 3.6	18.7 \pm 2.0
% fat	31.7 \pm 5.6	37.3 \pm 8.0
% carbohydrate	47.8 \pm 6.1	47.1 \pm 7.8

*each meal analyzed individually using Nutri-Calc, ver. 5.40; PCD Systems, Penn Yan, New York; reported here in abbreviated form.

Appendix Table 5. Two way ANOVA with repeated measures. The first value in the table represents the overall difference between injectors, the second item represents the difference (both injectors together) over time, and the third item represents the interaction between injector type and time. Significant differences between injectors are implied by differences ($p < 0.05$) in the injector and/or in the interaction items.

- A-5-1 Heart rate & change in heart rate
- A-5-2 Right & left pupil diameter
- A-5-3 Change in right & left pupil area
- A-5-4 Accommodation (right & left eyes)
- A-5-5 Atropine concentrations (RRA & RIA)
- A-5-6 Salivary secretion & blood 2-PAM concentration

HEART RATE

	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN ERROR	3864887.90658 47224.75132	1 19	3864887.90658 2485.51323	1554.97	0.0000		
2	INJECTOR ERROR	1737.10658 4026.91974	1 19	1737.10658 211.94314	8.20	0.0100		
3	TIME ERROR	94088.86842 25002.97368	18 342	5227.15936 73.10811	71.50	0.0000	0.0000	0.0000
4	IT ERROR	9405.76842 11704.70526	18 342	522.54269 34.22428	15.27	0.0000	0.0000	0.0000

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.2048	0.2602
4	0.2866	0.4062

ELAPSED TIME : 88.760 SECONDS

CHANGE IN HEART RATE

	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN ERROR	94806.49284 22943.25430	1 19	94806.49284 1207.53970	78.51	0.0000		
2	INJECTOR ERROR	1546.12643 5859.33849	1 19	1546.12643 308.38624	5.01	0.0373		
3	TIME ERROR	94092.72997 24997.10330	18 342	5227.37389 73.09095	71.52	0.0000	0.0000	0.0000
4	IT ERROR	9392.74879 11706.84661	18 342	521.81938 34.23055	15.24	0.0000	0.0000	0.0000

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.2047	0.2601
4	0.2865	0.4059

ELAPSED TIME : 88.760 SECONDS

RIGHT PUPIL DIAMETER

SOURCE		SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN	17897.14735	1	17897.14735	919.22	0.0000		
	ERROR	369.92904	19	19.46995				
2	INJECTOR	2.43835	1	2.43835	1.33	0.2623		
	ERROR	34.70915	19	1.82680				
3	TIME	264.51490	17	15.55970	39.53	0.0000	0.0000	0.0000
	ERROR	127.12371	323	0.39357				
4	IT	10.81590	17	0.63623	1.66	0.0485	0.1213	0.0763
	ERROR	123.65160	323	0.38282				

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

GREENHOUSE-GEISSER	HUYNH-FELDT
0.3028	0.4287
0.4232	0.7070

ELAPSED TIME : 88.760 SECONDS

LEFT PUPIL DIAMETER

SOURCE		SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN	18290.16001	1	18290.16001	981.23	0.0000		
	ERROR	354.16193	19	18.64010				
2	INJECTOR	5.92235	1	5.92235	3.10	0.0944		
	ERROR	36.29404	19	1.91021				
3	TIME	226.06424	17	13.29790	29.27	0.0000	0.0000	0.0000
	ERROR	146.75882	323	0.45436				
4	IT	7.50890	17	0.44170	1.38	0.1415	0.2125	0.1689
	ERROR	103.03971	323	0.31901				

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

GREENHOUSE-GEISSER	HUYNH-FELDT
0.3420	0.5097
0.4384	0.7491

ELAPSED TIME : 88.760 SECONDS

CHANGE IN RIGHT PUPIL AREA

ERROR TERM	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN ERROR	34254.36478 8411.01614	1 19	34254.36478 442.68506	77.38	0.0000		
2	INJECTOR ERROR	1731.66048 7803.85884	1 19	1731.66048 410.72941	4.22	0.0541		
3	TIME ERROR	16551.30016 9662.16956	17 323	973.60589 29.91384	32.55	0.0000	0.0000	0.0000
4	IT ERROR	748.48901 9465.58148	17 323	44.02877 29.30521	1.50	0.0915	0.1771	0.1381

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

ERROR TERM	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.2964	0.4161
4	0.3844	0.6073

ELAPSED TIME : 88.760 SECONDS

CHANGE IN LEFT PUPIL AREA

ERROR TERM	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN ERROR	33900.75092 8794.49942	1 19	33900.75092 462.86839	73.24	0.0000		
2	INJECTOR ERROR	2178.82823 4975.09165	1 19	2178.82823 261.84693	8.23	0.0095		
3	TIME ERROR	14474.13466 11141.40106	17 323	851.41969 34.49350	24.68	0.0000	0.0000	0.0000
4	IT ERROR	560.24563 7922.97971	17 323	32.95563 24.52935	1.34	0.1636	0.2379	0.2052

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

ERROR TERM	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.3445	0.5153
4	0.3914	0.6245

ELAPSED TIME : 88.760 SECONDS

ACCOMMODATION (RIGHT EYE)

ERROR TERM	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN ERROR	130453.51759 7826.64019	1 19	130453.51759 411.92843	316.69	0.0000		
2	INJECTOR ERROR	35.48514 477.27392	1 19	35.48514 25.11968	1.41	0.2493		
3	TIME ERROR	697.85962 2332.81757	15 285	46.52397 8.18532	5.68	0.0000	0.0037	0.0022
4	IT ERROR	94.86411 2575.29183	15 285	6.32427 9.03611	0.70	0.7843	0.4699	0.4789

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

ERROR TERM	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.1635	0.1895
4	0.1033	0.1108

ELAPSED TIME : 88.760 SECONDS

ACCOMMODATION (LEFT EYE)

ERROR TERM	SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN ERROR	150869.01833 20861.89712	1 19	150869.01833 1097.99459	137.40	0.0000		
2	INJECTOR ERROR	77.77126 506.88655	1 19	77.77126 26.67824	2.92	0.1040		
3	TIME ERROR	1348.48848 3739.25118	15 285	89.89923 13.12018	6.85	0.0000	0.0029	0.0019
4	IT ERROR	372.75749 8247.97970	15 285	24.85050 28.94028	0.86	0.6114	0.3910	0.3949

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

ERROR TERM	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.1333	0.1490
4	0.0862	0.0899

ELAPSED TIME : 88.760 SECONDS

ATROPINE CONCENTRATION (RRA)

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN	24360.41538	1	24360.41538	340.59	0.0000	
	ERROR	1358.95226	19	71.52380			
2	INJECTOR	95.56611	1	95.56611	0.86	0.3655	
	ERROR	2112.07051	19	111.16161			
3	TIME	6967.01794	18	387.05655	33.63	0.0000	0.0000
	ERROR	3935.81111	342	11.50822			
4	IT	615.98938	18	34.22163	4.49	0.0000	0.0000
	ERROR	2608.55690	342	7.62736			

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

GREENHOUSE-GEISSER	HUYNH-FELDT
0.1746	0.2133
0.3151	0.4646

ELAPSED TIME : 88.760 SECONDS

ATROPINE CONCENTRATION (RIA)

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F	TAIL PROB.	GREENHOUSE GEISSER PROB.	HUYNH FELDT PROB.
1	MEAN	34064.62121	1	34064.62121	310.20	0.0000	
	ERROR	1976.66105	18	109.81450			
2	INJECTOR	100.23789	1	100.23789	2.03	0.1711	
	ERROR	887.86034	18	49.32557			
3	TIME	6397.37985	18	355.40999	57.48	0.0000	0.0000
	ERROR	2003.31162	324	6.18306			
4	IT	346.46500	18	19.24806	7.70	0.0000	0.0000
	ERROR	810.43790	324	2.50135			

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

GREENHOUSE-GEISSER	HUYNH-FELDT
0.1143	0.1292
0.2033	0.2616

ELAPSED TIME : 88.760 SECONDS

CHANGE IN SALIVARY SECRETION

1	2	3	4
MEAN	INJECTOR	TIME	IT
ERROR	ERROR	ERROR	ERROR
2053147.64527	4674.30376	811206.39803	35573.75980
113749.61394	76886.23104	274157.75920	212368.68005
1	1	18	18
19	19	342	342
2053147.64527	4674.30376	45067.02211	1976.31999
5986.82179	4046.64374	801.63087	620.96105
342.94	1.16	56.22	3.18
0.0000	0.2959	0.0000	0.0000
		0.0000	0.0083
			0.0024

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

ERROR TERM	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.2897	0.4121
4	0.3042	0.4416

ELAPSED TIME : 88.760 SECONDS

BLOOD 2-PAM CONCENTRATION

1	2	3	4
MEAN	INJECTOR	TIME	IT
ERROR	ERROR	ERROR	ERROR
9684.69603	1.38297	2429.84286	68.02573
399.84484	160.68716	549.00592	318.02544
1	1	18	18
19	19	342	342
9684.69603	1.38297	134.99127	3.77921
21.04447	8.45722	1.60528	0.92990
460.20	0.16	84.09	4.06
0.0000	0.6904	0.0000	0.0000
		0.0000	0.0012
			0.0001

EPSILON FACTORS FOR DEGREES OF FREEDOM ADJUSTMENT

ERROR TERM	GREENHOUSE-GEISSER	HUYNH-FELDT
3	0.1786	0.2193
4	0.3225	0.4803

ELAPSED TIME : 88.760 SECONDS

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